Complementation by Restricted Phage T1

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The ability of restricted phage T1_{hr} to complement stocks of T1_{am} mutants carrying the P1 modification has been tested in mixed infection of *Escherichia coli* B(P1). Of the 18 genes tested, 15 could be complemented to give successful infection of approximately 10 to 25% of the cells, and 2 other genes consistently gave at least 5% complementation. The progeny phage produced in these infections was predominantly of the *hr*⁺*am* genotype. A mutant in gene 12 could be complemented very poorly, if at all. With one double mutant stock, the complementation obtained was nearly as good as the more poorly complemented of the two corresponding single mutants.

When a phage infects a restricting bacterium, part of the DNA is degraded to acid-soluble fragments within several minutes (1, 8, 13). Nevertheless, some of the genetic information from the restricted phage persists in a form that can enter into genetic recombination (see reference 6 for summary).

The functional capacity of the information introduced by certain restricted phages has been studied by testing the ability of the restricted phage to complement a related, non-restricted phage carrying a nonsense mutation. The results vary considerably, depending on the phage and the mutation being studied.

In the case of T-even phages, phage with nonglucosylated DNA (designated as T*) are restricted in *Escherichia coli* B. Hattman (8) found that T*2 and T*6, but not T*4, are able to complement T4_{am}122 (gene 42: dCMP hydroxymethylase). None of the three is able to complement T4_{am}82 (gene 44: mutant unable to synthesize DNA). Since the infection of strain B by any one of the three T* phages by itself results in the synthesis of significant amounts of dCMP hydroxymethylase (7, 8), the inability of T*4 to complement T4_{am}122 is rather paradoxical.

In the case of λ , the restriction by *E. coli* K of phage propagated on *E. coli* C, designated as λ .C, is due to the absence of a host-specific pattern of methylation at the 6-position of a small proportion of adenine residues in the phage DNA (see reference 2 review). Terzi (15) found a great variation in the ability of λ .C to complement various λ .K *sus* mutants in the infection of *E. coli* K. Several mutants were readily complemented by restricted phage,

whereas several others were not, with a tendency for late genes (plus gene N) to be poorly complemented.

In the same study, Terzi (15) found that after infection by λ .C alone, very little RNA was produced that would hybridize with phage DNA.

Bacteria lysogenic for phage P1 restrict both λ and T1 if these phages have been propagated on nonlysogenic hosts. This restriction is due to a methylation pattern that is specified by P1 and is independent of the methylation pattern specified by chromosomal genes of the host (2). Klein (9) tested the ability of T1.K, infecting starved cells of strain K(P1), to complement several T1_{am} mutants in simultaneous infection. Two of the mutants were complemented moderately well; these were mutants in genes 16 and 18 (required for head biosynthesis). (See reference 5 for gene function in T1.) Lower levels of complementation (a few percent) were observed with mutants in gene 10 (tail biosynthesis) and genes 13 and 15 (head biosynthesis). The lowest complementation was seen with a mutant in gene 2 (required for DNA synthesis).

As is true for λ , little or no T1-specific mRNA was detected after infection of a restricting host (11).

In none of the above studies was a considerable number of genes tested under comparable experimental conditions. It is therefore quite difficult to draw conclusions about reasons for the differences between different genes in terms of what happens to restricted DNA in vivo. We made a systematic study of the ability of restricted T1 to complement one amber mutant in each of the known T1 genes.

The results show that nearly all of the T1 genes can complement at least moderately well in this kind of experiment. Thus, most of the genome of a restricted T1 particle seems to be available for at least a moderate amount of transcription in a mixed infection with nonrestricted phage.

MATERIALS AND METHODS

Phage stocks. The phage used here included T1hr, propagated on *E. coli* B (designated as T1hr.B) and a series of amber mutants propagated on *E. coli* KB3(P1) (designated as T1am.P). The origin of the amber mutants, a map, and a preliminary characterization of the function of each gene have been described previously (5).

Bacterial stocks. The *E. coli* strains used are listed in Table 1, along with their relevant characteristics. When used as a mixed plating culture, the mixture strains of CS 100 and CS 100/1,t will be referred to as CS+CS/1, and the mixture of strains B and B/1,t will be referred to as B+B/1.

Media. Nutrient broth (NB) was used for the growth of bacteria. NB with 0.5% NaCl added (NB-NaCl) was used in the phage growth tube. NB-NaCl-KCN (0.01 M KCN added) was used to wash the cells after infection to remove unattached phage.

Complementation test. The desired quantities of T1hr.B and T1am.P were added to chilled suspensions of log-phase *E. coli* B(P1) in NB. After approximately 5 min of preattachment in the cold, the tubes were transferred to a 37 C water bath for 5 min, then chilled in an ice bath and diluted with 5 volumes of chilled NB-NaCl-KCN. Modifications of this attachment regime in some experiments are indicated below. Unattached phage were removed by three cycles of centrifugation and resuspension in cold NB-NaCl-KCN; the first supernatant was assayed on the mixed plating cultures CS+CS/1 for calculation of phage attachment. The final suspension was assayed for yielding cells on two assay systems: CS+CS/1 and B+B/1. A sample of the same suspension was diluted 10⁴-fold into a growth tube containing NB-NaCl at 37 C and incubated for 45 min, whereas the remainder of the suspension was again centrifuged, and the supernatant was assayed so as to allow correction of the yielding cell assay for any remaining unattached phage. (This correction was usually negligible.) At the

end of the incubation period, the progeny phage in the growth tube were assayed on the same two mixed-plating cultures. The results of all assays are calculated on the basis of the number of doubly infected cells, calculated from the viable cell assay and from the multiplicity of infection (MOI) of the two phage types, assuming that they are distributed according to the Poisson distribution.

The transmission index was measured in at least one experiment with each mutant by infecting a parallel sample of B(P1) cells with the appropriate T1am stock and plating the infected cell suspension on the CS+CS/1 mixture. The index was obtained by dividing the number of plaques (which were uniformly turbid, as expected) by the number of infected cells.

Decay of complementing ability. In these experiments, T1hr.B was added to the desired number of chilled attachment tubes as in the complementation test, but only one tube (the zero delay tube) received T1am.P. After the preattachment period, all tubes were transferred to the 37 C bath (time taken as zero). T1am.P was added to each tube after the desired delay period, and the tube was chilled and processed 4 min later as described above. The zero delay tube received 4 min of incubation at 37 C. The same kind of procedure was used in experiments in which T1am.P was added first.

RESULTS

In these experiments, complementation is inferred from the analysis of the phage produced when B(P1) is infected dually by T1hr.B and by T1am.P. Simple complementation would produce a pure burst of T1am hr⁺ progeny. Potentially, however, other processes may occur. Marker rescue, as studied in T1 by Drexler and Christensen (4), would produce either T1am hr or T1am⁺hr⁺. The latter, if produced, would be expected to be able to replicate since the am⁺ gene of the restricted phage would be incorporated into the genome of the unrestricted parent. Facilitation of the replication of the restricted parent in mixed infection might be termed "phage rescue," and would produce progeny phage of the genotype T1am⁺hr. Drexler and Christensen (4) observed

TABLE 1. Bacterial strains used

Strain	Relevant characteristics	Origin and/or reference
B B/1,t	Nonpermissive for T1am Nonpermissive; resistant to T1hr ⁺ ; sensitive to T1hr	E. Lennox Isolated in this laboratory
B(P1)	Nonpermissive; restricts T1.B	Isolated in this laboratory; P1 phage from G. Bertani
KB3	Permissive for T1am	H. Drexler; reference 10
CS100	Permissive	A. Doermann; reference 12
CS100/1.t	Permissive; resistant to T1hr ⁺ ; sensitive to T1hr	Isolated in this laboratory

very little of this in their experiments. We have distinguished to a considerable extent between complementation, marker rescue, and phage rescue by the analysis of the progeny phage and of the yielding cells on each of two mixed plating cultures, the one amber-permissive (CS+CS/1) and the other nonpermissive (B+B/1). Some representative results are shown in detail in Table 2.

Consider first the progeny phage. The total progeny are measured by the sum of all plaques on the CS+CS/1 mixture. Complementation, as noted above, will produce *T1am hr⁺* progeny, which will form turbid plaques on the CS+CS/1 mixture, but none on the B+B/1 mixture. The difference between these two numbers thus will give the number of progeny that are of the genotype expected from complementation. In both experiments, it is clear that the majority of phage are of this type, which leads us to conclude that complementation was the major process responsible for the production of phage in these experiments. This preponderance of *T1am hr⁺* progeny was a feature of all the experiments reported here, with the values ranging from 65 to 93%. The lower values were generally associated with those experiments in which the burst size was small.

Other general features of this kind of experiment can be seen in results in Table 2. Phage of the genotype expected from phage rescue, *T1am⁺hr* (measured as clear plaques on the

B+B/1 mixture), are in the smallest number, whereas the two genotypes that would result from marker rescue are intermediate in number. These are *T1am⁺hr⁺* (measured as turbid plaques on the B+B/1 mixture) and *T1am hr* (measured as clear plaques on CS+CS/1 minus clear plaques on B+B/1).

The analysis of the yielding cells is more complex, since a single cell can potentially release more than one genotype. In fact, it would be expected that a cell in which complementation occurred, allowing the replication of the *T1am* parent, would have an enhanced probability for marker rescue, in which case the cell could release either a mixture of *T1am hr⁺* and *T1am⁺hr⁺* (and so give a turbid plaque on B+B/1 as well as one CS+CS/1) or a mixture of *T1am hr⁺* and *T1am hr* (and thus give a sectoried plaque on CS+CS/1). Indeed, when infected cells are plated, these classes of plaques do represent a larger fraction of the total plaques than is the case when progeny phage are plated.

For simplicity, we have taken the sum of turbid and sectoried plaques on CS+CS/1 as the measure of the number of cells yielding phage by complementation. In many of the experiments, a substantial number of these cells were also capable of making plaques on B+B/1, and were thus yielding *T1am⁺* phage, but as noted above, the preponderance of *T1am hr⁺* in the progeny makes us believe that complementa-

TABLE 2. Representative complementation results

Input (phage and MOI)	Analysis of output (plaques per dually infected cell)										
	Yielding cells						Progeny phage				
	Plating culture	Clear	Turbid	Sec-tored	Total	Comple-mentation index ^a	Clear	Turbid	Total	Comple-mentation progeny ^b	Comple-mentation burst size ^c
T1hr.B (1.1) and T1am30.P (0.8)	CS+CS/1	0.18	0.39	0.059	0.63	0.45	1.5	13.0	14.0	11.0	24.0
	B+B/1	0.084	0.27	0.015	0.37		0.36	2.4	2.8		
T1hr.B (2.0) and T1am45.P (3.3)	CS+CS/1	0.017	0.16	0.025	0.20	0.18	0.084	0.56	0.64	0.43	2.4
	B+B/1	0.005	0.063	0.008	0.07		0.025	0.13	0.16		

^a Complementation index, (turbid plus sectoried plaques on CS+CS/1)/dually infected cell. See text.

^b Complementation progeny, (turbid plaques on CS+CS/1 – turbid plaques on B+B/1)/dually infected cell. See text.

^c Complementation burst size, complementation progeny/complementation index.

tion was the primary event, and that marker rescue was largely secondary, and dependent on complementation.

Effects of variation in the MOI of the restricted phage. It might be suspected that each incoming restricted phage might have a particular probability of being expressed, so that a gene dose effect would be seen if the MOI of the restricted phage were to be varied while holding the MOI of the *am* phage constant (Table 3). With T1am30, it can be seen that the expectation was realized; the number of cells yielding by complementation is approximately proportional to the number of restricted phage attaching. A similar result was found in experiments with T1am19 (data not shown). With T1am15, however, the results were quite different (Table 3): in this case, the number of cells yielding by complementation was proportional to the number of infected cells, rather than to the infecting restricted phage. Additional phages beyond the first do not increase the probability that complementation will occur. A similar result was seen with T1am10 (data not shown). A particular T1am mutant gives consistent results.

We have no explanation for the different behavior on the different genes of the restricted phage; some showed a gene dose effect and some did not. Perhaps a full knowledge of the function of the various genes would make these results understandable, but no generalization may be made based on the preliminary information now available.

Results with the collection of T1am mutants. To survey the ability of the various genes of the restricted phage to function, we have carried out complementation experiments with one mutant in each of the 18 genes of T1 that have so far been identified. In these experiments, the aim (not always achieved) was to use

an MOI of approximately 2 for each phage; in most of the experiments, the MOI of T1hr.B was from 1 to 3 and that of T1am.P was 1.5 to 4. Since the differing results with different genes did not allow any regular normalization of the results to compensate for the differences in the MOIs, the results are reported here simply on the basis of the complementation index. Also reported are the complementation burst sizes.

A summary of the results for all mutants tested except T1am37 (gene 12) are shown in Table 4. A few comments are perhaps in order about these results. There is a fair amount of scatter in the data, especially for some of the mutants, and it is probably fair to characterize the results with most of the mutants by saying that they give a complementation index of approximately 0.1 to 0.25, without placing any confidence on the apparent differences between them. Furthermore, among the four mutants giving the highest average complementation indices, three also have high transmission indices, so that if the complementation index is corrected by subtracting the transmission index, these three mutants are similar to most of the rest. Nevertheless, there may be real differences between the complementing ability of the different genes. The value obtained with T1am30 is consistently high, in spite of the low transmission index. On the low side, there are T1am15 (gene 5) which gave an average complementation index of 0.09 in the three experiments, and T1am5 (gene 2), with an average complementation index of 0.08 in five experiments.

In a few cases, the transmission index was high enough to cause some concern. In gene 7, T1am46 combined the highest complementation index measured with a high transmission index. Another mutant in the same gene, T1am35, gave a low transmission index and a

TABLE 3. *Effect of variation in the MOI of T1hr.B on the complementation of T1am.P*

Amber mutant	MOI		Yielders ^a	Yielders per dually infected cell	Yielders per infecting T1hr.B
	T1hr.B	T1am.P			
T1am30 (gene 18)	8.8	6.4	7.2×10^7	0.32	0.037
	2.3	7.8	1.8×10^7	0.090	0.035
	0.66	8.6	2.0×10^6	0.019	0.014
	0.23	7.8	1.2×10^6	0.027	0.025
T1am 15 (gene 5)	9.4	5.0	1.6×10^7	0.047	0.005
	2.6	4.3	9.2×10^6	0.029	0.011
	0.89	4.9	7.9×10^6	0.040	0.026
	0.11	6.6	1.5×10^6	0.042	0.042

^a Yielders by complementation, turbid plus sectorized plaques measured on CS + CS/1.

moderate complementation index, similar to that seen with most of the mutants. In gene 9, T1am13 also showed a high transmission index in two measurements but both were below the range found in five measurements of the complementation index. In all of these cases, the complementation burst size was consistently greater than 1, in contrast to the transmission burst size which in all of the experiments re-

ported here was approximately one phage per yielding cell.

With the mutant in gene 12, the problem of the transmission was the most serious, and the results are summarized in Table 5. Whether measured in terms of the number of yielding cells or in terms of the burst size, the complementation results were only marginally greater than the results with transmission alone. Unfortunately, we do not have any other mutants in this gene. Of all the genes, it seems that gene 12 is complemented the most poorly by a restricted phage.

Effect of delayed addition of T1am.P phage. In studying the persistence of the ability of restricted lambda to complement various amber mutants, Terzi (15) found a considerable variation between different genes. For example, the ability to complement an R mutant disappeared with a half life of approximately 5 min. The corresponding value for K mutants was approximately 2 min, whereas the activity toward N mutants, although initially very low, showed little change with incubation for up to 8 min.

On the other hand, Klein (9) in his experiments with T1 found no decline within 15 min in the ability of the restricted phage to complement T1am3 (gene 2) or T1am7 (gene 17), unless an inhibitor of RNA synthesis was present during the interval between infections.

In Klein's experiments (9), the host bacteria were starved before infection. Table 6 presents the results of a series of experiments done under our experimental conditions. With the possible exception of the gene 4 mutant, it can be seen that any loss of complementing ability for the various genes seems generally to be slow. In no case does there seem to be any significant loss within 4 min, and in some cases even the 8- or 16-min results are very similar to those with simultaneous infection. Thus, these genes of the restricted phage retain their ability to function

TABLE 4. Complementation by T1hr.B of various T1am mutants

Gene	Amber mutant	Transmission index ^a	No. of expt	Complementation index ^b (range)	Complementation burst size ^b (range)
1	am16	0.10	3	0.34-0.39	17-19
2	am5	0.03	5	0.05-0.12	9.2-16
3	am41	0.004	3	0.17-0.24	3.6-20
4	am23	0.05	3	0.13-0.19	25-52
5	am15	0.01	3	0.03-0.17	7.3-7.9
6	am18	10 ⁻⁴	3	0.12-0.20	15-40
7	am46	0.21	3	0.32-1.0	2.9-25
	am35	0.02	1	0.22	9.5
8	am32	0.02	3	0.18-0.31	6.3-21
9	am13	0.17, 0.18	5	0.25-0.40	11-40
10	am19	10 ⁻⁴	3	0.05-0.24	3.3-9.6
11	am9	0.06	3	0.19-0.29	2.9-11
12	am37 ^c				
13	am10	0.002	3	0.07-0.23	5.8-2.6
14	am45	0.006	3	0.18-0.22	2.3-29
15	am11	0.01	4	0.09-0.20	5.4-5.8
16	am4	10 ⁻⁴	3	0.16-0.34	19-28
17	am7	0.002	3	0.16-0.22	25-42
18	am30	10 ⁻⁴	3	0.22-0.42	11-28

^a Transmission index, proportion of B(P1) cells yielding phage after infection by T1am.P alone. With the exception of T1am13, this was measured in only one experiment in each set. See text for further comment.

^b See Table 2 for calculation of complementation index and complementation burst size.

^c See Table 5 for results.

TABLE 5. Complementation and transmission results with T1am37.P

Expt	Transmission			Complementation			
	MOI T1am37.P	Transmission index ^a	Transmission burst size ^b	MOI		Complementation index ^c	Complementation burst size ^c
				T1am37.P	T1hr.B		
1	4.2	0.15	1.0	4.7	1.9	0.25	2.6
2	8.8	0.14	1.6	9.9	4.4	0.17	4.8
3	6.7	0.35	0.63	6.8	2.8	0.32	2.5

^a See Table 4 for calculation.

^b Phage yield per yielding cell.

^c See Table 2 for calculation.

for a time that is significant compared with the latent period of the phage.

Effects of delayed addition of the restricted phage. Terzi (15) proposed that the reason that the several late-functioning lambda genes were complemented only poorly by a restricted phage is that the gene would be critical only late in the latent period, and the ability of the restricted phage to function would thus have been lost before this critical period. If this were so, then delaying the addition of the restricted phage should increase its ability to complement late genes. Table 7 shows the results with several T1 genes, including both genes that presumably function late and genes that presumably function early. The results were roughly similar to those cited above with a delay in the addition of the amber phage: little change within 4 min, with sometimes a drop by 8 min (certainly a late

time in the T1 life cycle). In no case was there an increase.

Experiments with a double am mutant. Taken one at a time, 17 of the 18 genes tested in the experiments above are being effectively expressed in from about 8 to about 35% of the cells. Are the different genes being expressed in different cells of the population, or is there a significant fraction of cells in which perhaps many genes are being expressed? To gain information on this point, several experiments were carried out with the double mutant T1am5am11. This double mutant was selected because the two mutations are near the opposite ends of the phage map, because one of the genes is complemented rather poorly (making the test more sensitive), and because we happen to have this double mutant in our collection (kindly provided by H. Drexler).

The average complementation index for T1am5 is 0.085 (five experiments) and for am11 0.13 (three experiments), so the double mutant would be expected to have a complementation index in the neighborhood of 0.01 if the two genes were being expressed independently. The actual result in four experiments was 0.06 (range 0.031 to 0.11). Whether it is judged by the mean values or by the extreme values, the double mutant is only slightly below the lower of the two single mutants. Table 8 shows results of these experiments, including an experiment in which the MOI of the restricted phage was varied and another experiment in which the addition of the am phage was delayed by 8 min; in these experiments also, the complementation of the double mutant gave results very similar to similar experiments with single mutants. We conclude that if a given cell expresses one gene

TABLE 6. Effect of the delayed addition of T1am.P on its ability to be complemented by T1hr.B

Gene	Amber mutant	Complementation index ^a when the addition of T1am.P was delayed for			
		0 min	4 min	8 min	16 min
4	T1am23	0.15	0.14	0.08	0.02
		0.13		0.04	
8	T1am32	0.32	0.30	0.16	0.13
		0.18		0.20	
10	T1am19	0.05	0.10	0.12	
		0.07	0.07	0.06	
11	T1am9	0.19	0.13	0.07	
13	T1am10	0.07		0.08	0.06
14	T1am45	0.22	0.21	0.14	
18	T1am30	0.29	0.40	0.12	

^a See Table 2 for calculation.

TABLE 7. Effect of the delayed addition of T1hr.B on its ability to complement T1am.P

Gene	Amber mutant	Complementation index ^a when the addition of T1hr.B was delayed for		
		0 min	4 min	8 min
1	T1am16	0.39	0.44	
2	T1am5	0.11	0.17	
6	T1am18	0.20	0.16	
		0.12	0.092	0.035
9	T1am13	0.30	0.40	
		0.40	0.19	
		0.36	0.28	0.15
14	T1am45	0.18	0.18	
15	T1am11	0.060	0.044	
17	T1am7	0.22	0.15	

^a See Table 2 for calculation.

TABLE 8. Complementation studies with double am mutant

Expt no.	MOI of T1hr.B	Genotype of T1.P	Complementation results	
			Yield- ing cells	Burst size
1	3.0	T1am5am11	0.11	12
2	1.5	T1am5am11	0.031	12
	1.9	T1am5	0.045	9.2
	2.0	T1am11	0.091	4.5
3	2.7	T1am5am11	0.036	9.6
	0.25	T1am5am11	0.031	7.1
4	2.2	T1am5am11	0.064	8.4
	4.2	T1am5am11 (added after 8-min delay)	0.042	6.2

of a restricted phage, it has a fairly high probability of also expressing a distantly linked gene, and that this property is not dependent on multiple infection by the restricted phage, nor is there any marked time-dependent dissociation of the expression of two genes.

DISCUSSION

When T1 infects a restricting host, a majority of the phage DNA is degraded to acid-soluble fragments within 5 min (13). Little or no T1-specific mRNA can be detected after infection of a restricting host (11). How then are we to understand the considerable amount of complementation by restricted T1, initially shown by Klein (9) and considerably extended by the results reported here?

First, both in the case of T1 and λ , restriction-induced degradation production stops well short of completion (1, 13). Freshman et al. (6) have summarized the evidence that a significant amount of the genetic information from a restricted phage is preserved, although it is in a state in which it neither functions nor replicates. The results presented here with the double mutant T1am5am11 suggest that the preserved fraction does not consist predominantly of small fragments, randomly distributed among the population of infected cells, although part of the preserved fraction may be in the form of fragments. Drexler (3, 17) has provided evidence that complete T1 genomes do persist, at least in a small proportion of restricting cells.

One explanation for the complementing ability shown by restricted T1 would be to assume that the transcription from the DNA of the unrestricted phage of a gene that provides a positive regulator of transcription is required before any transcription of the surviving fraction of restricted genomes can occur. (Perhaps the restricted genome, even though its genetic information has not been destroyed, is in an altered physical state in which this gene cannot be transcribed by the host RNA polymerase.) In such a case, unrestricted amber mutants in this gene would not be complemented. Little is known about the control of transcription in T1.

Of course, if the restriction nuclease always acted at a site within a particular gene, mutants in that gene would not be complemented. However, the P1 restriction nuclease seems to cut unmodified DNA molecules more or less randomly, although the lack of methylation at the specific modification site is a requirement for the enzyme to act at all (10).

In these experiments, the mutant in gene 12

showed the least ability to be complemented. From Figurski and Christensen (5), we can see that gene 12 is at the left end of a block of genes required for phage head biosynthesis, and is thus strategically located to have a regulatory function. On the other hand, gene 12 may include a site of particularly frequent attack by the P1 nuclease, or for some other reason be nontranscribable even if most of the genes of the restricted phage are activated by the unrestricted co-infecting phage.

In infection by restricted λ , of the six genes tested in simultaneous infection (15), only *susR* (a lysis gene) could be complemented at a level comparable similar to that seen here with most of the T1 genes. Mutants in the late genes C, G, E, and K, as well as the control gene N, were poorly complemented (2% or less). Several other mutants were tested only by a procedure in which infection by the restricted phage preceded the infection by the *sus* mutant by 10 min; mutants in genes J, O, P, and Q were all readily complemented, whereas mutants in genes A, B, and H were poorly complemented. A mutant in gene I gave intermediate results.

In summary, most of the genes of a restricted T1 show considerable ability to complement unrestricted amber mutants in mixed infection. This provides support for the persistence of a significant fraction of the genetic information introduced into the cell by the restricted phage. The reasons for differences in the complementing activity between different phages or between different genes of the same phage remain unexplained.

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