Ligase-Defective Bacteriophage T4

II. Physiological Studies

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The timing of the suppression of gene 30 (deoxyribonucleic acid ligase) mutations by *rII* mutations was studied by temperature shift-down experiments with a temperature-sensitive *rII* mutation. The *rII* function must remain inactivated for about 5 to 8 min at 37°C for suppression to occur, thus making suppression an early function. This result is in agreement with the timing of expression of other *rII* functions. A gene 30 defect can also be overcome by replacing the Na⁺ cation in the growth medium with the Mg²⁺ cation, a result similar to the relief of the lethality of *rII* mutations in lambda lysogens. Prior infection with bacteriophages T3 or T7, which produce their own deoxyribonucleic acid ligases, can also partially overcome the lethality of gene 30 mutations.

During the course of experiments to probe the role of bacteriophage T4 gene 30 (deoxyribonucleic acid [DNA] ligase) in the genetic determination of frameshift mutation rates (19), several novel observations were made concerning the suppression of the lethal effects of gene 30 mutations by *rII* mutations, by manipulation of the cation composition of the growth medium, and by co-infection with bacteriophages T3 or T7.

The T4 *rII* cistrons appear to control a membrane modification in the host cell brought about by a mechanism which remains obscure, but which affects many parameters of T4 development (1, 3, 11, 12, 21-23, 26-28). The *rII*⁺ functions are mostly expressed early in phage development (6, 16, 25, and references therein). One of these *rII*⁺ functions brings about an increased requirement for gene 30 function, with the result that *rII* mutations act as suppressors of gene 30 mutations, probably either by directly or indirectly decreasing endonuclease activity (2, 9, 17, 18, 20, 30). The timing of this suppression was of importance in experiments in which the proflavine-induced reversion of *rII* frameshift mutations was examined in a gene 30 *ts* (temperature-sensitive) background (19), and temperature shift-down experiments reported here demonstrate that *rII* function must remain inactivated only early in T4 development for suppression to occur. These results are complementary to previous reports (20) along similar lines using temperature shift-up experiments.

Mutational defects in the *rII* cistrons are themselves lethal in lambda lysogens. This

lethality can be lifted, however, by manipulation of the cation concentration of the medium (specifically by eliminating monovalent cations and increasing the concentration of divalent cations) or by adding certain polyamines, amino acids, or sucrose (4, 5, 11, 12, 28). During experiments to probe the optimal conditions for the suppression of ligase defects by *rII* mutations, it was observed that the lethality of gene 30 mutations could also be partially lifted by replacing Na⁺ by Mg²⁺ cations.

Suppression of gene 30 mutations by *rII* mutations is strong even when the gene 30 mutation consists of an amber lesion. The host ligase apparently substitutes for the T4 ligase under these conditions, as shown by elegant experiments in which gene 30 suppression by *rII* mutations was reversed by host ligase mutations (14, 20). It was therefore of interest to determine whether other ligases besides that of the host cell could also substitute for the T4 ligase. It was already well known that infection by bacteriophages T3 or T7 largely overcomes by superinfection with bacteriophage T4, even when several minutes elapse between the T-odd and the T-even infection. During this interval, the two T-odd phages express their own ligase functions (24). It was therefore possible to show that the two T-odd ligase functions can also substitute for the T4 ligase function.

MATERIALS AND METHODS

Most materials and methods are described in the first paper of this series (19) and in references therein.

Phage and bacteria. The T4 gene 30 mutants *amH39*, *amC104*, *amE605*, *tsA80*, and *tsB20* were ob-

tained from the Caltech collection (10). The *T4rII* mutations *rUV200* and *rSM122* and wild-type T4D, T3, and T7 are from the Drake collection (7, 8); *rSM122* is temperature sensitive for growth on lambda lysogens. T7*am13* is a ligase amber mutant (24). *Escherichia coli* CR63 was used for growing stocks of the amber mutants. N953 is an *E. coli* K-12 strain wild type for ligase function, and N1325 is the double mutant *lop8-lig2* which is substantially defective in ligase function (14).

Temperature shift-down experiments. Stocks of the gene 30 mutant *amH39* were screened for pseudorevertants which could plate on BB cells at 43 C but not at 32 C and which, when backcrossed to T4D, yielded both *amH39* and a temperature-sensitive *rII* segregant, the latter defined by its plaque morphology on B cells and its ability to grow on lambda lysogens at 32 C but not at 43 C. Temperature shift-down experiments were performed by infecting BB cells at 37 C with one of these double mutants, *amH39-rK4*, at a multiplicity of infection (MOI) of 5. At intervals from 4 to 13 min, the complexes were diluted into broth at 25 C, and lysis was terminated with chloroform at 120 min. Burst sizes were compared with those obtained with T4D alone grown under identical conditions.

Manipulation of cation concentrations. Both phage stocks and cells were grown and diluted in 1% tryptone broth (Difco) containing 0.08 M NaCl (to permit adsorption). Log-phase cells were infected with an MOI of 5, and at 2 min anti-T4 serum was added to inactivate unadsorbed phage. At 5 min the complexes were diluted 1,000-fold into 1% tryptone broth containing 0.005 M tris(hydroxymethyl)amino-methane (Tris) buffer, pH 7.2, plus various other components. At 60 min chloroform was added to complete lysis. Burst sizes were measured on BB cells or, in the case of *amH39*, on CR63 cells.

Co-infection experiments. Log-phase cells at 37 C in L broth were infected with bacteriophage T3 or T7 at an MOI of 5. At 1 min the complexes were superinfected with bacteriophage T4 at an MOI of 5. At 3 min anti-T4 serum was added to inactivate unadsorbed phage. At 9 min the complexes were diluted 1,000-fold. At 60 min chloroform was added to complete lysis. The lysates were then treated with anti-T7 serum to inactivate most of the T7 progeny. The burst size was measured on CR63 cells by using the initial infected cell concentration as a base.

RESULTS

Temperature shift-down experiments. The temperature-sensitive *rII* lesion in *amH39-rK4* is capable of suppressing the gene 30 mutation at 37 C or higher, but not at 25 C. If infection occurs initially at 37 C, but the temperature is abruptly dropped to 25 C at various times thereafter, the time during which the *rII* function must remain inactive for the gene 30 defect to be suppressed can be determined. If the temperature is shifted down at any time before about 6 min after infection, the burst

size remains very small (less than 2% of the *amH39-rK4* control burst size) (Fig. 1). When the shift-down occurs after about 10 min, however, a normal burst size is obtained. The promotion of lethality brought about by the *rII*⁺ function in a gene 30 mutant is therefore an early function of bacteriophage T4.

Cation rescue of gene 30 mutants. Replacement of Na⁺ in the growth medium by Mg²⁺ permits the completion of a growth cycle by T4*rII* mutants in a lambda lysogen (12). Table 1 shows that high concentrations of Mg²⁺ also produce substantial increases in the average burst sizes of both amber and *ts* gene 30 mutants. The wild-type burst size, however, is not achieved, and variable burst sizes are produced by 0.12 M Mg²⁺ with different gene 30 mutants. (This variation is considerably greater than the concomitant variation in wild-type burst sizes in different experiments.) The combination of Na²⁺ and Mg²⁺ cations produces a smaller burst size than does Mg²⁺ alone, indicating an active role for the Na⁺ cation in promoting the lethality of gene 30 mutations.

It was observed previously (12, 29) that replacement of monovalent cations by 0.1 M Tris buffer, or even by 0.15 M sucrose, partially relieved the lethality of *rII* mutations in lambda

FIG. 1. Temperature shift-down effects on burst size of *amH39-rK4* growing in BB cells. Infection was initiated at 37 C, and the shift-down to 25 C was performed at the indicated time. Relative burst sizes were obtained from parallel shift-down experiments using T4D.

TABLE 1. Burst sizes of T4 mutants growing in supplemented broth^a

Mutant	Affected gene	Supplement			
		None	0.08 M NaCl	0.12 M MgCl ₂	Both
<i>amH39</i>	30	0.0040(2) ^b	0.035(8)	30(5)	10.5(3)
<i>amH39^c</i>	30	0.0058(1)	0.027(2)	15(2)	4.0(1)
<i>amC104</i>	30	0.011(1)	0.11(1)	23(1)	10.9(1)
<i>amE605</i>	30	0.0014(1)	0.015(1)	1.3(1)	1.1(1)
<i>tsA80</i>	30	0.20(1)	0.032(3)	7.3(3)	
<i>tsB20</i>	30		0.013(2)	0.21(2)	
<i>rUV200</i>	<i>rIIA</i>		0.0024(5)	31(4)	
<i>rSM122</i>	<i>rIIA</i>		0.011(1)	0.18(1)	
T4D	None	2.4(1)	103(2)	103(2)	83(1)

^a KB host cells were used except where indicated. Experiments were run at 37 C with the wild-type and the amber mutants (including *rUV200*), and at 43 C with the temperature-sensitive mutants (including *rSM122*).

^b Numbers in parentheses indicate the number of measurements performed to determine the given average.

^c BB host cells were used.

lysogens. This result has been confirmed: the burst size of *rUV200* in KB cells was increased from 0.0024 (in 0.08 M Na⁺) to 0.78 by 0.10 M Tris buffer, and to 1.9 by 0.15 M sucrose. The burst size of *amH39*, however, was 0.0040 in unsupplemented broth, 0.035 in 0.08 M NaCl, 0.0024 in 0.10 M Tris buffer, and 0.11 in 0.15 M sucrose, indicating little or no relief even by 0.15 M sucrose.

The relationship between burst size and Mg²⁺ concentration was explored for the wild type and for both *rII* and gene 30 mutants. Figure 2 shows burst sizes normalized to the maximal burst size observed with each genotype. (The maximal burst sizes were 190 for the wild type, 77 for the *rII* mutant, and 31 for the gene 30 mutant.) The optimal Mg²⁺ concentrations are clearly different for the rescue of *rII* and gene 30 mutants, being about 0.06 M and about 0.14 M, respectively. Burst sizes decreased precipitously above 0.14 M Mg²⁺, even for the wild type.

Co-infection experiments. The DNA ligases of T4 and *E. coli* can, under appropriate circumstances, be functionally equivalent for T4 replication (14, 20). Even the T3 or the T7 ligase can substitute for T4 replication, to a limited extent (Table 2). Relief of gene 30 mutational lethality by T3 or T7 is only moderately efficient in B and BB cells, however, and is even less efficient in the two K-12 strains tested. The tests with *E. coli* N953 and N1325 (carrying a host ligase defect) suggest that the functional state of the host ligase makes little additional difference to the co-infection effect. In other experiments not documented here, the time of T4 infection compared to the time of T3 or T7 infection was varied from -3

FIG. 2. MgCl₂ effects on relative burst sizes of wild-type T4D, *rII* mutant *rUV200*, and gene 30 mutant *amH39*.

to +4 min; the greatest effect was observed at +1 min.

It would appear from Table 2 that the T7 rescue effect depends critically upon the functional state of the T7 ligase gene. This conclusion is limited, however, by the fact that T7*am13* adsorbs very poorly and is relatively less sensitive than the parental T7 strain to our anti-T7 sera. The extent of co-infection was therefore difficult to measure, and could have been small. The fact that burst sizes

TABLE 2. Effect of co-infection with T3 or T7 on the growth of ligase-deficient T4

Host cell	Helper phage	T4 ^a genotype	T4 burst size	Relative burst size
BB	None	<i>amH39</i>	0.0043	0.00003
	T7 <i>am13</i>	<i>amH39</i>	0.0069	0.00005
	T7	<i>amH39</i>	8.1	0.063
	T7	WT	128	
B	None	<i>amH39</i>	0.017	0.00007
	T7 <i>am13</i>	<i>amH39</i>	0.028	0.00011
	T7	<i>amH39</i>	19	0.074
	T7	WT	257	
N953	None	<i>amH39</i>	0.64	0.0058
	T7	<i>amH39</i>	1.7	0.015
	T7	WT	110	
N1325	None	<i>amH39</i>	0.0069	0.00007
	T7	<i>amH39</i>	1.8	0.013
	T7	WT	143	
BB	None	<i>amH39</i>	0.0062	0.00004
	T3	<i>amH39</i>	11.6	0.078
	T3	WT	148	
BB	None	WT	308	2.1
	None	<i>amC104</i>	0.020	0.005
	T3	<i>amC104</i>	9.8	0.24
	T3	WT	40.3	
BB	None	WT	103	2.6
	None	WT		

^a WT, wild type.

were increased by over 1,000-fold by wild-type T7, however, but less than 2-fold by T7*am13*, still strongly suggests that the T7 ligase gene is specifically responsible for the observed rescue.

DISCUSSION

DNA ligase function appears to be required continuously for normal DNA replication during T4 development (29). Since the *rII* function is expressed early in T4 development, whereas DNA replication and the associated action of DNA ligase only occur later (31), it was important to determine the interval during which *rII* function must remain inactivated for *rII* suppression of a gene 30 defect to occur. A recent report, also employing a temperature-sensitive *rII* mutation, used temperature shift-up experiments (from 30 to 41°C) to measure this interval (20). It was observed that the shift-up must occur within the first 10 to 20 min for suppression to occur. Using temperature shift-down experiments, it is reported here that the *rII* function must remain inactivated for 5 to 10

min at 37°C for suppression of a gene 30 defect to occur. Since T4 development is about twice as fast at 30°C as at 37°C, the two experiments are completely complementary, and both indicate that suppression of gene 30 defects by *rII* mutations occurs early in T4 development. This result should be compared to the timing of two other *rII* functions: the lethality of *rII* mutations in lambda lysogens is established early, as shown by both shift-up (25) and shift-down (21) experiments, and the ability of infected cells to be subjected to lysis inhibition is also an early function (6). All of the known *rII* functions therefore become established early in T4 development.

The rescue of gene 30 defects by replacing monovalent cations by divalent cations has not been as extensively explored here as has the corresponding rescue of *rII* defects. It is clear, however, that the rescue of the two functions occurs under somewhat different conditions, particularly with respect to the optimal Mg²⁺ concentration. The chemical mechanism of reactivation remains obscure in both cases, but several workers have speculated that the reactivation of *rII* defects occurs at the cell membrane or else acts to overcome membrane defects by overcoming leakage phenomena (4, 5, 11, 28). This speculation seems reasonable, but it is perfectly possible that *rII* and gene 30 rescue proceed by independent mechanisms.

Experiments reported here which show a limited rescue of gene 30 mutational defects by co-infection with bacteriophage T3 or T7 were stimulated by earlier reports that the S-adenosylmethionine-cleaving enzyme synthesized after T3 infection could act during subsequent T2 infection to produce unmethylated T2 progeny (13), and that T7 produces its own DNA ligase, for which an amber mutant is available (24). Limited rescue of a gene 30 defect (maximum efficiency 24%) could be obtained, and variations on the present methods (for instance, using ultraviolet-irradiated T3 or T7 plus longer intervals before superinfection) might improve the method. The main purpose of these experiments, however, was to demonstrate the circumvention of T4 ligase defects by ligases beyond that of the host itself, and to extend the observation of enzymatic cross-functioning between highly unrelated pairs of viruses. The present results suggest that many fruitful tests could be made of the functions elaborated by a virus such as T7, before mutants of these functions have been obtained, or even before they have been defined by appropriate enzymological studies,

simply by testing for the repair of defects in well characterized T4 genes.

The gene 30 mutant *amH39*, which was used in many of the experiments reported here, also contains a mutational defect in the gene determining α -glucosyl transferase (15). Rescue experiments by both Mg^{2+} cations and bacteriophages T3 and T7 have also been performed, however, by using the gene 30 mutants *amC104* and *amE605*, with results that reveal that the second mutational defect in *amH39* does not affect the present experiments.

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