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Mutagenic Effect of Temperature-Sensitive Mutants of Gene 42 (dCMP Hydroxymethylase) of Bacteriophage T4

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Certain temperature-sensitive mutants of gene 42 of bacteriophage T4 increase the reversion rates of some *rII* mutants in the same genome by about 4 to 10 times. This effect was usually found at 34 C, an intermediate permissive temperature, but not at 28 C.

Speyer and co-workers (6, 7) have shown that certain temperature-sensitive mutants of gene 43, the structural gene for T4 DNA polymerase (3, 8), cause an increased reversion rate of *rII* mutants in the same chromosome, and Drake and Allen (4) have demonstrated antimutator effects with selected gene 43 mutants. In the course of a study of temperature-sensitive mutants of gene 42 (2; P. K. Tomich and G. R. Greenberg, manuscript in press), we discovered that these mutants increased the reversion rates of a number of *rII* mutants.

Bacteriophage T4D, the temperature-sensitive mutants of gene 42 (*L13* and *LB1*) employed earlier (2), and the gene 45 mutant, *L159*, were originally from R. S. Edgar. The *rII* mutants, derivatives of T4B, were *HB118*, obtained from S. P. Champe, and *UV4*, *UV6*, *UV13*, *UV183*, *SM18*, and *SM94*, provided by John W. Drake. *Escherichia coli* K12(λ), strain 3350, from Allen Campbell was obtained through Myron Levine of this University. *E. coli* BB was from J. W. Drake, *E. coli* S/6 was from R. S. Edgar, and strain B was used earlier (2).

Double mutants carrying *rII* and temperature-sensitive lesions were constructed by using *E. coli* BB as the host cell. From each cross a number of separate recombinants were isolated. The number of these isolates ultimately analyzed is given as the figures in parentheses in Table 1. Single-plaque isolates were first tested for their temperature sensitivity by using *E. coli* S/6 as the indicator cell. Phage that failed to grow at 42 C were tested for their *rII* phenotype: ability to grow at 30 C on *E. coli* S/6 plates but not on *E. coli* K12(λ). Lysates were prepared from the double mutants by using *E. coli* BB as the host. Titers ranged from 2×10^{10} to 10^{11} phage/ml. The ratio of growth at 42 C to 30 C was 10^{-5} to 10^{-6} and, depending on the *rII* mutation, the ratio of growth on K12(λ) to B was 10^{-6} to 10^{-8}. Each isolate in a given cross was then tested for the reversion rate of its *rII* mutation. The figures shown in footnote *a* to Table 1 represent these separate reversion analyses.

Reversion studies were carried out by the method of Speyer (6) with slight modifications. A culture of 5 ml of *E. coli* BB at 5×10^7 to 6×10^7 cells/ml in nutrient broth was infected with about 1,000 phage. Tryptophan was added at 50 μg/ml prior to infection. At about 2 min after infection, the culture was diluted with 10 ml of growth medium, and then divided to provide triplicate analyses. The infection process was continued in a rotary shaker bath at 28 or 34 C, maintained to less than ±0.5 C for 3.5 h, and then terminated with chloroform. Cell debris was removed by low-speed centrifugation, and the number of phage was measured by plating dilutions in duplicate. Reversion analyses were carried out on at least two and sometimes up to nine separate plaque isolates of each mutant or double mutant.

The several *rII* mutations were tested for their reversion rates alone and as double mutants with either *tsL13* or *tsLB1* at two temperatures, both permissive, i.e., 28 and 34 C (Table 1). Reversion is expressed as the ratio of the phage titer on *E. coli* K12(λ) to that on *E. coli* B. The spontaneous reversion indices (K12(λ)/B $\times 10^{-8}$) for the individual *rII* mutants compare well with those given by Drake and Allen (4). In Table 1 an increased reversion rate of the double mutant theoretically occurs when the double to single ratio is greater than 1.0; however, varia-

TABLE 1. *Reversion of rII mutants*[a]

Phage	Reversion index × 10⁸ [b]			Double mutant/single mutant	
	28 C	34 C	Ratio: 34 C/28 C	28 C	34 C
rHB118	3.6 (2)	12 (2)	3.3		
rHB118/tsL13	5.1 (2)	5.9 (2)	1.2	1.4	0.5
rUV4	3.4 (2)	11 (2)	3.2		
rUV4/tsL13	3.5 (2)	2.7 (2)	0.8	1.0	0.25
rSM18	163 (2)	236 (6)	1.4		
rSM18/tsL13	198 (8)	932 (8)	4.7	1.2	4.0
rSM18/tsLB1	282 (6)	1,030 (9)	3.6	1.7	4.3
rSM18/tsL159[c]	67 (6)	267 (6)	4.0	0.4	1.1
rSM94	2.5 (2)	2.2 (2)	0.9		
rSM94/tsL13	2.2 (2)	7.1 (2)	3.2	0.9	3.2
rUV6	64 (3)	88 (3)	1.4		
rUV6/tsL13	102 (3)	377 (3)	3.7	1.6	4.3
rUV183	75 (4)	118 (4)	1.6		
rUV183/tsL13	55 (4)	290 (4)	5.3	0.7	2.4
rUV183/tsLB1	43 (4)	1,020 (7)	24	0.6	8.6
rUV13	9.7 (5)	8.7 (5)	0.9		
rUV13/tsL13	35 (6)	95 (8)	2.7	3.6	10.9

[a] The figures in parentheses are the number of individual plaque isolates used in the experiments. All values are averages as in the typical data shown below. No data have been omitted in these calculations and no jackpot yields (Drake and Allen, 1968) were observed. Each of the following figures is from a lysate prepared from a separate plaque isolate and represents the average titer of 3 lysates prepared from an infection (see Text). *UV13* at 28 C: 7.3, 9.1, 12, 7.2, 13 (av, 9.7); *UV13* at 34 C: 6.9, 12, 6.9, 6.9, 11 (av, 8.7). *UV13/L13* at 28 C: 25, 38, 65, 22, 37, 23 (av, 35); *UV13/L13* at 34 C: 40, 60, 138, 89, 115, 104, 104, 108 (av, 95). *UV183* at 28 C: 32, 106, 80, 82 (av, 75); *UV183* at 34 C: 117, 177, 97, 83 (av, 118). *UV183/LB1* at 28 C: 18, 15, 89, 51 (av, 43); *UV183/LB1* at 34 C: 585; 1,050; 2,300; 477; 1,020; 804; 893 (av; 1,020). *SM18* at 34 C: 241, 171, 288, 262, 192, 296 (av. 236). *SM18/L13* at 34 C: 2,193; 1,123; 1,265; 530; 467; 128; 1,040; 705 (av, 932). *SM18/LB1* at 34 C: 797; 933; 1,287; 675; 926; 1,623; 1,200; 1,000; 789 (av; 1,030).

[b] Plaques formed with strains: K12 (λ)/B.

[c] Gene 45.

tions between 0.5 to 2 are not considered as significant. Increased reversion with temperature is shown by the 34 C to 28 C column.

Clearly, at 34 C the reversion rates of several of the double mutants were higher than the values obtained with the single *rII* mutants. The double mutants *rUV183/tsLB1* and *rUV13/tsL13* showed the largest increases. This is corroborated by footnote *a* to Table 1 which shows the reversion rates for each of the separate plaque isolates in these and in several other cases. At 34 C, three- to fourfold increases were shown with *rSM18/tsL13*, *rSM18/tsLB1*, *rSM94/tsL13*, and *rUV6/tsL13* over their single mutant counterparts. At 28 C, however, the values for the double to single mutants were close to 1.0. At this temperature, *rUV13/tsL13* was the only double mutant showing an increase in reversion rate over the single mutant. The reversion rates of the *rII* mutants *rHB118* and *rUV4* were not increased by the presence of the *tsL13* mutation at either 28 or 34 C. Without further study it cannot be stated whether *tsL13* has an antimutator effect on *rUV4*.

The reversion rate of *rUV183/tsL13* at 34 C was only 2.4 times that of *rUV183* alone, whereas *rUV183/tsLB1* showed a value which was 8.6 times that of the single mutant control. At 28 C, however, the two double mutants were both identical to the control. We confirmed by hydroxylamine and bromouracil tests that *rUV183* exhibited an AT → GC reversion pattern (4). It appears then that *tsLB1* may act differently than *tsL13* or *tsLB3* (see below) on the reversion of *rUV183* (Table 1). By contrast, with *rSM18*, a transversion mutant (4), the observed effects by *tsL13* and *tsLB1* were the same. *tsL159*, a mutant of gene 45, an uncharacterized DO gene (unable to synthesize DNA), did not increase the reversion rate of *rSM18*. We have deferred discussion of the nature of the reversion patterns of the other *rII* mutants employed. References to these patterns are available in the paper by Drake and Allen (4).

We conclude that at 34 C the presence of certain *ts* mutants of gene 42 causes a significant increase in the reversion rate of some *rII* mutants but not in others. The reversion rates of some *rII* mutants were increased by a maximum of about 10 times, i.e., about 50 times less than the greatest increases found with gene 43 mutants (6, 7).

W. E. Williams and J. W. Drake at the University of Illinois, Urbana, have corroborated these observations by using the gene 42 mutant, *tsLB3*, and various *rII* mutants (W. E. Williams and J. W. Drake, personal communication). In some instances by a somewhat modified procedure they found increases in reversion rate up to about 20-fold. *tsLB3* appears to cause mainly HMC → T transitions in *rII* reversions since *rUV183* did not show an increased reversion. These workers also demonstrated mutagenic effects at lower temperatures than we employed (see also *rUV13/tsL13*, Table 1), and they also found that the mutation rate does not increase as the phage yield is reduced at higher temperatures.

The synthesis of hydroxymethyl dCMP ceases at 42 C in infection by *tsL13* (P. K.

Tomich and G. R. Greenberg, manuscript in press) and would be expected to be decreased at intermediate, partially permissive temperatures in some relationship to the rate of DNA synthesis. Nevertheless, it seems unlikely that the increased mutagenesis seen here at 34 C and in one instance at 28 C could arise as a result of a decreased supply of 5-hydroxymethyl dCTP. Thus, an analysis of the profiles of DNA synthesis versus temperature with various *ts* mutants of gene 42 showed that in *tsL13* infection, DNA synthesis is approximately at its maximum at 34 C (C.-S. Chiu and G. R. Greenberg, unpublished studies). Actually *tsLB1*-infected cultures show increased DNA synthesis up to 36 C, decreasing above that temperature (C.-S. Chiu and G. R. Greenberg, unpublished data). This conclusion is also supported by the lack of effect of lower phage yield at higher temperature on the mutation rate (W. E. Williams and J. W. Drake, personal communication). Thus, it is reasonable to conclude that the increased reversion shown by *rUV183* with *tsLB1* as compared to *tsL13* is due to the nature of the gene 42 mutation and not to a general lack of gene product.

Bernstein (1) has found that certain frameshift mutations in the *rII* region showed increased reversion in the presence of selected *ts* mutations of the DO genes 32, 43, and 44 and, in a less extensive series of tests, that mutations in the DO genes 30 and 45 showed no effect on one of the *rII* mutants. It may be mentioned that *rUV6* (Table 1) is a frameshift mutant (4). J. D. Karam (personal communication) has found that *ts* mutants in gene 62, a DO gene, also cause small increases in the rate of reversion of certain *rII* mutations.

It has been suggested that DNA polymerase itself has a role either in determining the fidelity of copying the template (6, 7) or in editing by its 3′ → 5′ exonuclease activity (5). Since the structure of the DNA-synthesizing apparatus is unknown, the significance of both the enhanced effect of *ts* mutants of gene 42 and of other DO genes on the *rII* reversion rates obviously must await additional study.

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LITERATURE CITED

1. Bernstein, H. 1971. Reversion of frameshift mutations stimulated by lesions in early function genes of bacteriophage T4. J. Virol. **7**:460-466.
2. Chiu, C. S., and G. R. Greenberg. 1968. Evidence for a possible direct role of dCMP hydroxymethylase in T4 phage DNA synthesis. Cold Spring Harbor Symp. Quant. Biol. **33**:351-359.
3. DeWaard, A., A. V. Paul, and I. R. Lehman. 1965. The structural gene for deoxyribonucleic acid polymerase in bacteriophages T4 and T5. Proc. Nat. Acad. Sci. U.S.A. **54**:1241-1248.
4. Drake, J. W., and E. F. Allen. 1968. Antimutagenic DNA polymerases of bacteriophage T4. Cold Spring Harbor Symp. Quant. Biol. **33**:339-344.
5. Muzyczka, N., R. L. Poland, and M. J. Bessman. 1972. Studies on the biochemical basis of spontaneous mutation. I. A comparison of the deoxyribonucleic acid polymerases of mutator, antimutator, and wild type strains of bacteriophage T4. J. Biol. Chem. **247**:7116-7122.
6. Speyer, J. F. 1965. Mutagenic DNA polymerase. Biochem. Biophys. Res. Commun. **21**:6-8.
7. Speyer, J. F., J. D. Karam, and A. B. Lenny. 1966. On the role of DNA polymerase in base selection. Cold Spring Harbor Symp. Quant. Biol. **31**:693-697.
8. Warner, H. R., and J. E. Barnes. 1966. Deoxyribonucleic acid synthesis in *Escherichia coli* infected with some deoxyribonucleic acid polymeraseless mutants of bacteriophage T4. Proc. Nat. Acad. Sci. U.S.A. **56**:1233-1240.