THE EFFECT OF A BACTERIAL MUTATOR GENE UPON MUTATION RATES IN BACTERIOPHAGE T4[1]

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MUTATOR genes can be defined as entities that can be mapped on the chromosome and that induce mutations at other loci. Although genes of this type have been known in Drosophila for over 25 years (DEMEREC 1937; PLOUGH and HOLTHAUSEN 1937; GOLDSCHMIDT 1939, 1945; NEEL 1942; IVES 1943), it is only since their discovery in bacteria and viruses (TREFFERS 1954; MIYAKE 1960; SPEYER 1965) that an understanding of their action has begun to evolve.

Several mechanisms have been proposed for the action of a mutator gene. One is that the primary gene product of a mutator gene is an altered DNA polymerase which permits errors in replication. SPEYER (1965) has found that some of the temperature-sensitive mutants at the DNA polymerase locus in bacteriophage T4 have such mutator gene properties. Another hypothesis is that the mutator gene produces a mutagenic base analogue which is incorporated into the DNA of the cell. KIRCHNER (1960, also referred to in HAYES 1964) is reported to have found a fifth base in the chromatographic analysis of the bases of MIYAKE's mutator strain of *Salmonella typhimurium*, in which the mutator gene is closely linked to markers involved in base synthesis.

The research reported here constitutes an attempt to study the mechanism of action of a mutator gene. The gene chosen was that of TREFFERS, SPINELLI, and BELSER (1954) in *Escherichia coli*. The reasoning on which the experiments were based is as follows: if the mutator gene makes an endogenous mutagenic substance such as that assumed in Salmonella, this substance might be able to induce mutations in virulent phage, such as T4, growing in cytoplasm containing it. If, however, the mutator gene makes an imprecise DNA polymerase, it should not affect the phage, because (a) the T-even phages make their own DNA polymerase (KORNBERG 1960), and (b), when the viral gene controlling the structure of this enzyme has mutated to an inactive form, the bacterial DNA polymerase is unable to substitute for the defective phage enzyme in making bacteriophage DNA (DEWAARD, PAUL, and LEHMAN 1965; WARNER and BARNES 1966).

MATERIALS AND METHODS

Bacterial stocks: All stocks were derived from *E. coli* K-12 strains 58–278(λ) and its derivative containing the mutator gene, 58–278(λ)M*; both were kindly supplied by DR. H. P. TREFFERS.

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Strains 58–278C and 58–278M*C are the above stocks cured of bacteriophage lambda. Strains 58–278(λ)S and 58–278CS are mutants of 58–278(λ) and 58–278C, respectively, that are immune to 0.1 mg per ml. of streptomycin sulfate.

Bacteriophage stocks: T4D, presumably wild-type, was supplied through the courtesy of Dr. S. Champe. It is resistant to 0.25 μg per ml of acriflavine neutral and thus must carry a mutation at the *ac* locus. It is, however, sensitive to 2.5 μg per ml of the same drug and is thus wild-type at the *q* locus (Susman and Edgar, referred to in Pratt, Stent, and Harriman 1961). T4*rII* AP129, also supplied by Dr. Champe, is an *rII* mutant induced by 2-aminopurine. T4*rII* FCO+, kindly donated by Dr. S. Brenner, is an *rII* mutant induced by acridine yellow.

Media: Tryptone broth: Tryptone, 10 g; NaCl, 8 g; Na citrate, 2.3 g; distilled water, 1 liter. Streptomycin broth: tryptone broth supplemented with 0.1 mg per ml of streptomycin sulfate U.S.P. (Eli Lilly). Acridine streptomycin broth: streptomycin broth supplemented with 2.5 μg per ml of acriflavine neutral (Nutritional Biochemical). All base agar was made from the appropriate broth supplemented with 20 g of Bacto-agar and 1.3 g of glucose per liter; all top agar was made from the appropriate broth supplemented with 7.0 g of Bacto-agar per liter.

Method for measuring mutation rates in T4: This technique is based on a modification of the fluctuation test for bacterial mutation rates (Luria and Delbrück 1943). To measure mutation rates at the *q* locus, one ml of a suspension containing about 10^8 phage per ml was added to 99 ml of cold broth. At time zero, 20 ml of logarithmically growing, permissive *E. coli* cells, either 58–278(λ) or 58–278(λ)M*, at a concentration of about 10^8 per ml, were added. One-ml aliquots of the mixture were distributed to each of 70 tubes in a 37°C water bath and the tubes were incubated for approximately 2 hours to allow mutants to arise. Then, to each of ten control tubes, nine ml of cold streptomycin broth were added and the tubes kept cold until their titer could be determined (less than 1 hour). To assay for acriflavine resistant mutants, 3 ml of acridine streptomycin top agar containing *E. coli* 58–278(λ)S were added to each of the 60 remaining experimental tubes. Each was poured onto an acridine streptomycin agar plate and incubated overnight at 37°C. The ten control tubes were then assayed on *E. coli* 58–278(λ)S in streptomycin agar to determine the average total number of infective centers per tube. The next day, the 60 plates were scored for the presence of mutant plaques. If fewer than 12, or more than 54, of the plates lacked mutant plaques, the experiment was discarded because the inherent error was too high (Lea and Coulson 1948). Mutation rates were calculated according to the formula $x = -[(\ln 2)(\ln p_0)]/N_t$ where x is the mutation rate, p_0 is the fraction of tubes lacking mutants, and N_t is the average total number of infective centers per tube.

The above procedure was modified for the determination of the rate of reversion of *rII* mutants in the following manner: the initial permissive cells were either 58–278C or 58–278M*C(λ carrying strains are not permissive for *rII* mutants); revertants were assayed on *E. coli* 58–278(λ)S in streptomycin top agar; the control tubes were assayed on *E. coli* 58–278C in streptomycin agar.

The titers of T4D on 58–278(λ), 58–278(λ)M*, and 58–278(λ)S were the same, as were those of the *rII* mutants on 58–278C, 58–278M*C, and 58–278CS.

RESULTS

Mutation rates at three different loci were determined—*q*, *rIIB*, and *rIIA*. The wild type at the *q* locus, q^+, is sensitive to 2.5 μg per ml of acriflavine neutral. The mutant *q*, can grow in this concentration of the drug when a second mutation *ac* is present (Susman and Edgar, referred to in Pratt, Stent, and Harriman 1961), as is the case with the T4D phage stock used.

Mutation rates at this locus were measured for T4D grown on 58–278(λ) and on 58–278(λ)M*. The results are summarized in Table 1. The first eight experiments are shown above the line; in five of these, 58–278(λ) was used as the host, and, in three, 58–278(λ)M*. The eight experiments indicated a difference

TABLE 1

Mutation rates for T4D ac $q^+ \rightarrow$ *T4D* ac q

(a) Grown on 58–278(λ)	(b) Grown on 58–278(λ)M*
($x_a \times 10^{-7}$)	($x_b \times 10^{-7}$)
10.5	...
2.68	...
8.43	15.5
7.35	52.2
12.0	34.2
21.5	88.9
9.12	17.6
4.96	11.1
6.48	8.92
12.2	130
9.25	23.6
6.74	43.4
$\bar{x}_a{}^* = 9.21 \times 10^{-7}$	$\bar{x}_b = 42.54 \times 10^{-7}$
$\text{SD}_a\dagger = 4.81 \times 10^{-7}$	$\text{SD}_b = 39.15 \times 10^{-7}$
Student's t-test.	Total data: t‡ = 2.94
	P < .005
	Paired data: t = 2.25
	.05 < P < .025

* $\bar{x}$ = Mean mutation rate.
† SD = Standard deviation.
‡ t = Student's value.

between mutation rates at the same locus when the phage were grown in the presence or absence of the mutator gene on otherwise co-isogenic stocks, but the variation between different experiments was high. Therefore, seven paired experiments were run in which the same phage were grown in the presence and absence of the mutator gene at the same time. The data from the paired experiments are shown below the line. The mean for the total data on mutation rates at the *q* locus for stocks grown on 58–278(λ) was 9.21×10^{-7} with a standard deviation of 4.81×10^{-7}, and for stocks grown on 58–278(λ)M* was 42.54×10^{-7}, with a standard deviation of 39.15×10^{-7}. The mean difference in the paired data was 36.28×10^{-7}, with a standard deviation of 42.66×10^{-7}. Student's t-tests on both the paired and the unpaired data show these differences to be significant at the 5% level.

Considering the large variance in mutation rates in the experiments on both 58–278(λ) and 58–278(λ)M*, it is significant to note that in each of the paired experiments, the mutation rate of phage grown on 58–278(λ)M* was higher than that of phage grown on 58–278(λ). If there were no difference between hosts, one would expect the mutation rate on 58–278(λ)M* to be higher than that on 58–278(λ) half the time. The probability of the mutation rate on 58–278(λ)M* being higher than that on 58–278(λ) in seven consecutive experiments, if there were no difference in the mutation rate, is $(\frac{1}{2})^7$ or 0.78%. The data indicate

therefore, that the mutator gene in the bacterial host does affect mutation rates in the phage, but the variance of the measurement in the *q* system is too large to allow any study of the precise action of the mutator. Therefore, the *rII* system was chosen to study the system further.

An *rII* mutant cannot grow on *E. coli* lysogenic for bacteriophage lambda (BENZER 1955). The first such mutant examined here was *rII* FCO$^+$, a point mutation in the *B* cistron induced by acridine yellow and known to be revertable. Reversion rates from *rII* FCO$^+$ to *rII*$^+$ were measured for T4*rII* FCO$^+$ grown on 58–278C and on 58–278M*C. The results are shown in Table 2. The mean reversion rate for the *rII* FCO$^+$ mutant when grown on 58–278C was 7.67×10^{-8} with a standard deviation of 5.31×10^{-8}, and the mean reversion rate for the same mutant when grown on 58-278M*C was 7.99×10^{-8} with a standard deviation of 7.02×10^{-8}. There is no significant difference between these two means.

The third set of experiments was run on another *rII* mutant T4*rII* AP129. This revertable mutant is located in the *A* cistron of the *rII* locus. Reversion rates from *rII* AP129 to *rII*$^+$ were measured for T4*rII* AP129 grown on 58–278C and on 58–278M*C. The data are shown in Table 3. In this case, the mean reversion rate for *rII* AP129 grown on 58–278C was 4.61×10^{-8} with a standard deviation of 2.69×10^{-8}, and the mean reversion rate for the same locus on 58–278M*C was 66.5×10^{-8} with a standard deviation of 11.9×10^{-8}. The difference between these two sets of data is highly significant and the variances were both quite low.

DISCUSSION

The data presented here indicate that the mutator gene in the bacterial host increases mutation rates in bacteriophage genes, and, furthermore, that this activity show specificity with respect to the particular characters involved. Specificity of this mutator gene in reverting mutations in the tryptophan synthetase *A* gene of *E. coli* has been reported recently by YANOFSKY, COX, and HORN (1966). Their data indicate that M* induces adenine-thymine to cytosine-guanine transversions.

As explained in the introduction, the fact that TREFFER's gene does affect T4

TABLE 2

*Mutation rates for T4r*II *FCO*+ → *T4r*II+

(a) Grown on 58–278C	(b) Grown on 58–278M*C
($x_a \times 10^{-8}$)	($x_b \times 10^{-8}$)
3.05	9.93
7.25	7.72
16.22	2.49
8.39	18.73
3.45	1.10
$\bar{x}_a = 7.67 \times 10^{-8}$	$\bar{x}_b = 7.99 \times 10^{-8}$
$SD_a = 5.31 \times 10^{-8}$	$SD_b = 7.02 \times 10^{-8}$
Student's t-test: t = .16	
.45 > P > .40	

TABLE 3

*Mutation rates for T4*rII *AP129/T4*rII+

(a) Grown on 58–278C	(b) Grown on 58–278M*C
($x_a \times 10^{-8}$)	($x_b \times 10^{-8}$)
6.18	77.3
8.62	53.9
2.66	73.6
3.02	53.3
2.39	72.2
$\bar{x}_a = 4.61 \times 10^{-8}$	$\bar{x}_b = 66.5 \times 10^{-8}$
$SD_a = 2.69 \times 10^{-8}$	$SD_b = 11.0 \times 10^{-8}$
Student's t-test: $t = 12.21$	
$P << .0005$	

mutation rates is a strong indication that the mutagenic gene product is not an imprecise DNA polymerase and that it must be a substance which acts through the cytoplasm. It is compatible with the assumption that the gene product is an endogenous mutagen such as a fifth base similar to the one KIRCHNER is reported to have found in Salmonella (1960, referred to in HAYES 1964). If such a base exists, then it may be demonstrable in digests of DNA from phage grown on hosts containing the mutator gene.

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SUMMARY

Using a null-point technique, mutation rates have been determined for three loci in bacteriophage T4 grown on *Escherichia coli* stocks with and without TREFFER's mutator gene. The mutator gene raised both the forward mutation rate at the *q* locus and the reversion rate of the *rII* mutant induced by 2-aminopurine; the gene did not affect the reversion rate of another *rII* mutant induced by acridine yellow. It is concluded that the mutator gene probably produces an endogenous mutagen other than an imprecise DNA polymerase.

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