Second Printing 1966 / University of Texas Printing Division, Austin

MUTATOR FACTOR IN SALMONELLA TYPHIMURIUM[1]

T. MIYAKE[2]

Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, New York

Received October 26, 1959

SPONTANEOUS mutability is known to be considerably higher in strain LT-7 of *Salmonella typhimurium* than in strain LT-2. Observations, made by many workers in this laboratory, of frequencies of reversion in auxotrophs derived from LT-7 have shown wide variation in rate of reversion among individual mutants. This fact suggested that the laboratory's stock of strain LT-7 is heterogeneous with regard to spontaneous mutability, in other words, that some of the cells have higher mutability than others. In an experiment to test this possibility, the indicator was frequency of mutation from streptomycin sensitivity (*str-s*) to streptomycin resistance (*str-r*). Approximately 100 cells of strain LT-7 were plated on each of three nutrient agar plates, which were incubated overnight at 37°C; 240 colonies picked at random from these plates were resuspended in a small amount of saline and streaked on nutrient agar containing 100 μg/ml of streptomycin. After 20-hour incubation at 37°C, the streaks were observed to be of two types: 90 of them showed many *str-r* mutants, whereas 150 had very few or none. This test confirmed the probability that the LT-7 stock strain is heterogeneous, and indicated that about 40 percent of its cells carry a mutability factor which makes the *str-r* gene more mutable.

In order to find out whether this factor also affects the stability of other genes, 57 auxotrophic mutants were isolated from a strain derived from a colony which in the previous test had shown a high percentage of *str-r* mutation, and 51 auxotrophs from a strain derived from a colony having a low percentage of mutation. In the first group of auxotrophs, 87.7 percent had high reversion frequencies, whereas only 17.6 percent of the second group had high mutability. Thus it appears that the mutability factor effects the stability of a considerable proportion of different genes. To determine whether or not the mutability factor itself is stable, 90 colonies grown from a bacterial culture carrying the factor, and 90 from a culture not carrying it were compared by streaking on nutrient agar that contained streptomycin. All the streaks of the first lot and none of the second lot had a large number of *str-r* mutants.

The evidence of these experiments revealed that a stable mutability factor is responsible for high gene mutability in some cultures isolated from strain LT-7, and also that the strain is heterogenous, about 40 percent of its cells carrying the factor.

[1] Aided by a grant to M. DEMEREC from the American Cancer Society.

[2] Present address: Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

The next step in the analysis was to determine the nature of the factor, that is, whether it is a gene or some cytoplasmic constituent. This investigation was facilitated by the finding that *S. typhimurium* strains carrying the mutator readily hybridize with Hfr strains of *Escherichia coli* K-12. Since a similar mutator is known in *E. coli* (TREFFERS *et al.* 1954) and had been found to be a gene located fairly close to the *thr* (threonine requirement) locus, crosses were made between *S. typhimurium* having the genetic constitution mutator, *thr-61*, *lac*$^-$ (inability to utilize lactose) and *E. coli* HfrCS-101 having the constitution *met* (methionine requirement), *lac*$^+$. Equal volumes of overnight cultures of Salmonella and Escherichia were mixed, and 0.1 ml samples of the mixture were plated immediately on minimal-lactose agar supplemented with threonine (20 μg/ml). After 48-hour incubation at 37°C, *lac*$^+$ recombinants were picked, purified by single-colony isolation, and tested for *thr*$^+$ or *thr* and also for the presence or absence of the mutability factor by streaking on nutrient agar plates containing 100 μg of streptomycin per ml. Out of 65 *lac*$^+$ *thr*$^+$ recombinants, six (or 9.2 percent) did not carry the mutability factor, whereas all of 127 *lac*$^+$ *thr* recombinants did have the factor. Results of experiments with the same parental strains in which *thr*$^+$ recombinants were selected on minimal-glucose agar plates and tested for *lac*$^+$ or *lac*$^-$ and also for the presence or absence of the mutability factor, showed that three out of 61 (4.9 percent) of the *lac*$^+$ *thr*$^+$ recombinants did not carry the factor and four out of 125 (3.2 percent) of *lac*$^-$ *thr*$^+$ did not carry it.

These findings supply good evidence that the mutability factor is a gene, since it recombined in an expected fashion with the genes serving as markers in the crosses. It has been named mutator (*mut*). The results suggest that the order of the three genes tested is *mut—thr—lac*. According to SKAAR (BRYSON *et al.* 1955; SKAAR 1956), *mut* in *E. coli* is located to the right of *thr*; thus, if the two loci are homologous, the gene is located differently in the two species.

Experiments were made with ten alleles of the Salmonella *tryB* locus in order to compare their spontaneous mutability in genomes carrying *mut* and genomes carrying *mut*$^+$. By means of transduction, the *tryB* genes were introduced into the genomes of two *tryD* mutants, one of which (*tryD-7*) is *mut*$^+$ and the other (*tryD-29*) *mut*. Recombinants in which *tryB* replaced *tryD* were selected by plating on enriched minimal agar supplemented with indole, which supports the growth of *tryB* only. Since the *mut* locus is at some distance from the *try* region, it is never included in a *try*-carrying transducing fragment, and thus all recombinants retained the *mut* or *mut*$^+$ marker of the recipient bacteria. Twenty strains obtained in this way, each carrying one of the ten *tryB* genes in combination with either *mut* or *mut*$^+$, were tested for spontaneous mutability. Representative data from one test are given in Table 1. Three plates, each containing about 2×10^9 bacteria, were scored in a test. The results with *tryB-60* and *tryB-62* indicate that these two are stable auxotrophic genes; reversions were not observed on a total of 12 plates each with *mut* and 12 with *mut*$^+$. It seems likely that they originated through chromosomal aberrations. No reversions of *tryB-63* were

TABLE 1

Spontaneous mutability of different tryB *genes in the original genomes carrying* mut$^+$ *(or* mut *in the cases of* tryB-23, -67, *and* -68) *and when transferred to other genomes carrying* mut *(or* mut$^+$)

Allele	Revertants per plate (average three plates) Original	*mut*	*mut*$^+$	*mut/mut*$^+$
tryB-14	4.0	>1000	14.0	>71
-16	2.7	1147	8	143
-17	5.7	75	1.3	58
-18	0.3	1.7	0.3	6
-60	0	0	0	...
-62	0	0	0	...
-63	0	32	0	...
-23	155	148	0.3	493
-67	37	31	4	8
-68	1620	1620	6	270

observed on eight plates among the bacteria carrying *mut*$^+$, but 155 revertants were found on eight plates with *tryB-63 mut* cells.

In all seven of the *tryB* alleles that underwent spontaneous mutations when *mut*$^+$ was present, frequency of mutation was considerably increased by the presence of *mut*. The increase was not uniform, being six-fold in *tryB-18* and about 500-fold in *tryB-23*. The relative degree of increase in frequency does not depend on the relative frequency in the presence of *mut*$^+$, as is especially evident in the data for *tryB-23* and *tryB-67*. The action of the *mut* gene closely resembles that of various mutagenic agents (DEMEREC 1953) in that it affects the stability of different genes of a genome in a highly specific way.

SUMMARY

A mutator gene (*mut*) in *Salmonella typhimurium* affects the stability of all other markers of the genome with which it has been tested. The mutant form of the gene increases spontaneous mutability to different degrees in different alleles of the same locus; in this specificity of effect the action of *mut* is like that of various mutagenic agents. The high mutability observed in strain LT-7 is due to the presence of *mut* in about 40 percent of the cells of a population. The mutator gene is located to the left of *thr-leu* on the Salmonella chromosome.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to DR. M. DEMEREC and his associates at the Department of Genetics, Carnegie Institution of Washington, for invaluable advice, stimulating discussions, and encouragement during the course of this investigation. He particularly wishes to thank MISS AGNES C. FISHER for valuable help in the preparation of the manuscript.

LITERATURE CITED

BRYSON, V., P. D. SKAAR, H. DAVIDSON, J. HADDEN, and G. BOMPIANI, 1955 Genetic and biochemical studies of bacteria. Ann. Rept. Biol. Lab. 1954–1955: 16–22.

DEMEREC, M., 1953 Reaction of genes of *Escherichia coli* to certain mutagens. Symposia Soc. Exptl. Biol. **7**: 43–54.

SKAAR, P. D., 1956 A binary mutability system in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **42**: 245–249.

TREFFERS, H. P., VIOLA SPINELLI, and NAO O. BELSER, 1954 A factor (or mutator gene) influencing mutation rates in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **40**: 1064–1071.