GENETIC INSTABILITY WITH EPISOME-MEDIATED TRANSFER IN
ESCHERICHIA COLI

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ABSTRACT

GUNDERSEN, Wenche B. (Oslo University, Oslo, Norway), KAARE JYSSUM, AND SVERRE LIE. Genetic instability with episome-mediated transfer in Escherichia coli. J. Bacteriol. 83:516-623. 1962.— Hospital strains of Escherichia coli have been simultaneously screened for an increased mutation rate to streptomycin (Sm) resistance and to auxotrophy. Two strains were found with an increased mutation rate to Sm, but with no more than normal mutation rates in other loci tested. These loci included threonine, histidine, methionine, isoleucine-valine, resistance to tetracycline, and resistance to chloramphenicol.

The genetic determinant of the new type of instability of the Sm locus has been studied. In contrast to the Treffers mutator gene, the new mutator character was not found to follow the pattern of a chromosomal factor in genetic cross. The character was found to possess the properties generally ascribed to genetic determinants of an episomal nature: (i) a spontaneous loss of the character, enhanced by treatment with ultraviolet irradiation and acridine dyes; (ii) a loss which apparently is permanent; and (iii) a transfer to a mutator-negative cell by infection, both to a homologous mutator-negative mutant and to a K-12 strain. This infection is favored by the presence of the F factor in the donor.

Two distinct types of genetic instability have been described in Escherichia coli. One type comprises the Treffers strain, which is a wild-type K-12 with a mutator allele (Treffers, Spinelli, and Belse, 1954), the Harvard strain (Goldstein and Smoot, 1955), and strain no. 26 (Jyssum, 1960). These strains have in common an enhanced spontaneous mutation frequency in several independent genetic loci on the bacterial chromosome, including that of streptomycin (Sm) resistance. Salmonella typhimurium LT7 (Miyake, 1960) seems to have the same type of genetic instability. The only loci so far found not to be affected in the Treffers strain are a p-aminobenzoic acid locus (Bryson et al., 1955), a locus conferring resistance to the phage T2 (Treffers et al., 1954), and a cysteine locus (Treffers, personal communication). This type of enhanced mutation frequency is due to the action of a mutator gene located in the threonine-leucine region of the bacterial chromosome. The other type of instability is less understood; the strains of this type studied so far yield mutants only in the B3, methionine, leucine, and Sm resistance loci (Jyssum, 1960).

It has been the purpose of the present investigation to search for other types of genetic instability among hospital strains of E. coli, and to study the genetic nature of such instability.

MATERIALS AND METHODS

Bacterial strains. The organisms which were screened for increased mutation rates were isolated from patients. Only strains which conformed with the description of the species E. coli in Bergey’s Manual of Determinative Bacteriology (7th ed.) were used.

In comparative experiments, E. coli K-12 (wild-type revertant no. 1 M) with mutator allele was used (Treffers et al., 1954). This strain, as well as K-12 T 71, prototroph, M−, was obtained from H. P. Treffers, Yale University. In sexual conjugation experiments, the strains Hfr Cavalli (K 10), prototroph, Sm* and Hfr Hayes (3000), B−, Sm* were employed. These strains were provided by L. Gorini, Harvard Medical School. The F* K-12 strain W6, meth−, Sm*, lac+ was received from Ida Ørskov, Statens Seruminstitut, Denmark.

Media. Heart infusion broth and agar (Difco) were used as liquid and solid complete media. The minimal medium was prepared as described by Davis and Mingioli (1956). Agar (Difco) was used for the solid minimal medium. Glucose,
in a concentration of 0.1% in the liquid and 0.5% in the solid media, was used as the energy and carbon source. Amino acids and vitamins were kept in sterile solutions and added to the medium when growth factors were required.

Screening methods. Screening for any increased number of colonies resistant to Sm was carried out on agar plates containing 100 μg streptomycin per ml (Treffers et al., 1954). Screening for enhanced spontaneous auxotrophic mutation was performed by the penicillin technique described by Adelberg and Myers (1953), as previously used (Jyssum, 1960).

Genetic methods. Auxotrophic mutants were prepared by the improved penicillin technique described by Gorini and Kaufman (1960). Mutation rates to prototrophy were determined by the tube dilution method of Luria and Delbrück (1943). The mutation rate was calculated from the number of tubes showing growth after 3 days of incubation, and the number of organisms in the inoculum. The mutation rate to Sm resistance was determined likewise from the number of tubes which contained no resistant variants (Treffers et al., 1954). Colonies were scored for the presence of a mutator character with influence on the stability of the Sm resistance locus by means of Sm plates, either by streaking or by the replica plating method (Lederberg and Lederberg, 1952).

For sexual conjugation, the strains were grown separately in complete medium to the exponential phase and then mixed in equal proportions. The mating was allowed to take place for 150 min, whereupon the cells were centrifuged, washed, and spread in dilutions on selective media. After 2 to 3 days in the incubator, the recombinants were scored for nonselected markers. F− strains were rendered F+ by infection from the strain W6 meth−F+. The infecting strain and the acceptor strain, provided with appropriate genetic markers, were grown separately overnight in complete medium with shaking. The two strains were then grown to log phase in complete broth, and equal portions of each culture were mixed. After incubation at 37 C overnight, the mixture was centrifuged, resuspended in saline, and plated in dilutions on minimal plates with supplements which allowed the acceptor strain to grow. Colonies were picked from the growth obtained and checked for the complete set of genetic markers of the acceptor. Colonies which conformed with the requirements were provisionally tested for their F character by inoculation on a carpet of an appropriately marked F− strain which had been spread on plates permitting growth of recombinants only. Strains which gave a high number of recombinants were further checked for presence of the F character.

Ultraviolet (UV) irradiation. Two different lamps were used as sources of UV irradiation: a Hanovia Chromatolite Portable Ultra-Violet Lamp of 30 w and a Sterilelectric lamp of 15 w.

Chemicals. Amino acids were the products of Nutritional Biochemicals Corp., Cleveland, Ohio, or Mann Research Laboratories, Inc., New York, N. Y. Acriflavine was purchased from Matheson, Coleman & Bell, Cincinnati, Ohio.

Abbreviations. The following abbreviations are used for the genetic markers: Sm* = streptomycin sensitive; Smf = streptomycin resistant; ilva = isoleucine-valine; hi = histidine; tryp = tryptophan; leu = leucine; thre = threonine; meth = methionine; Mu = mutator factor; M = mutator locus of the Treffers strain; ad = adenine.

RESULTS

Search for strains with new types of genetic lability. Among 20 strains of E. coli which were simultaneously screened for a high mutation rate to Sm resistance and for any increased spontaneous auxotrophic mutation, 4 strains were found which were sufficiently different from the typical E. coli strain, as well as from the previously known strains with increased mutation rate, to merit a further investigation. These strains could be arranged in two groups. Two of the strains showed a high mutation rate to Sm resistance, while the yield of auxotrophic mutants was no more than that found in the normal E. coli strain. In the other two strains, the screening evinced an increased number of auxotrophic mutants, while the mutation rate to Sm resistance appeared to be normal. During this survey of E. coli strains, those showing a high mutation rate to Sm resistance as well as to auxotrophy (the pattern known from other strains with increased mutation rate) were not considered. The present paper is concerned with the genetic background of the type of increased mutation in one strain (no. 635) of the first group of new mutators.
General properties of strain 635. Strain 635, which was arbitrarily chosen for further study, was originally isolated from the urine of a patient. The strain was methionine-dependent, and was found to yield segregants which showed a no higher than normal mutation rate to Sm resistance. It was screened for mutation to Sm resistance and to auxotrophy as a prototrophic revertant. This strain was found to produce a colicin-like substance active against strains K-12 and B; it carries an unidentified temperate bacteriophage. Neither the colicin nor the phage has been found to bear any relation to the high mutation rate to Sm resistance. The phenomena of colicinogeny and lysogeny in strain 635 have not been investigated further. Strain 635 is resistant to the action of the generally transducing phage pIko.

Stability of the mutator character in strain 635. To explore the stability of the Mu character as an inheritable factor, strain 635 was grown in complete broth overnight, and inoculated on complete agar plates to ensure separate colonies. These colonies were scored for increased mutation rates to Sm resistance. Of 752 colonies of the prototroph which were scored in four separate experiments, 26 colonies (3.45%) had lost the high mutation rate. The percentages ranged from 1.4 to 4.6 in the separate experiments. Similarly, 13 colonies (5.4%) of 635 thre-hi among 240 tested had lost the Mu character. When these experiments are combined, a total of 39 of 992 colonies tested lost the Mu character. This gives a 3.9% loss in the colonies, ranging from 1.4 to 5.4% in the individual experiments. To determine whether the loss of the Mu character is a permanent one, 1298 colonies of a 635 Mu− were scored for revertants to Mu+. None was found, a fact which is taken to indicate that the apparent loss of this character is an actual one.

Isolation of auxotrophic mutants in strain 635. The Trefers strain, K-12 1 M, has been found to yield all kinds of auxotrophic mutants spontaneously. In contrast to this, strain 635 appears to have a very low frequency of spontaneous mutation to auxotrophy. By neither the penicillin technique described by Adelberg and Myers (1953) nor by the improved technique of Gorini and Kaufman (1960) has it been possible to recover spontaneous mutants in this strain. By UV irradiation and the latter screening method, however, auxotrophic mutants of all types desired have been easily obtained.

The generation time of strain 635 growing exponentially in a minimal medium has been found to be approximately 45 min. The time allotted for "pregrowth" (2 hr) and exposure to penicillin (90 min) for E. coli B (Gorini and Kaufman, 1960) was also favourable for strain 635. For the isolation of mutants of strain K-12, 3 hr pregrowth and 105 min exposure to penicillin were found necessary.

The yield of auxotrophic mutants in all amino acid requirements tested was abundant in the UV-irradiation experiments with strain 635. Indeed, so many mutants were recovered that only a few were regularly kept from each group. Although the purpose of this screening method was to isolate mutants with only one additional amino acid requirement in each separate experiment, the assays with strain 635 also yielded several mutants with growth requirements different from those expected. In some instances, mutants were recovered with more than one growth requirement acquired during the experiment. In most cases these mutants were discharged with no further investigation. In one case, however, when selecting for threonine requirement with a 635 prototroph as the original strain, 15 good thre− mutants were isolated. In addition to these, 16 mutants which grew on complete medium but not on threonine or minimal plates were further investigated. Of these strains, 2 were found to require adenine, 3 required methionine, and the remaining 11 strains seemed to require a combination of vitamins and amino acids. These strains were not subject to any further investigation. A few of the auxotrophic mutants prepared by this procedure turned out to be temperature sensitive. Several of the mutants had lost their Mu character, but the majority still possessed it.

Determination of mutation rates in various genetic loci of strain 635. The Trefers strain has been reported to yield spontaneously a high number of auxotrophic mutants, and the mutants thus obtained, as well as those obtained by UV irradiation, have a high spontaneous reversion rate to prototrophy.

The reversion rates of several of the auxotrophic mutants of strain 635 were determined in the manner described by Luria and Delbrück (1943). Some results of such reversion rate
determinations have been recorded in Table 1. It is an important feature of the experiments recorded that the reversion rate in the threonine locus in a strain where the Mu character is present does not significantly differ from the rate in a strain in which this character is absent. The results of the reversion rate experiments are as a whole taken to indicate that the Mu character has no influence upon the mutation rate of genetic loci other than that of Sm resistance. These results agree with the finding that it was impossible to recover spontaneous auxotrophic mutants in strain 635 by either of the penicillin techniques employed.

A determination of the mutation rate to Sm resistance proved difficult since it was too high for an exact calculation by the dilution method. It was found, however, to be higher than $2.8 \times 10^{-8}$. The rate has also been estimated very crudely by spreading a culture on Sm plates in different dilutions. Such experiments indicate that the rate is of the order of $10^{-4}$. Strain 635 has also been tested for an increased mutation to resistance against tetracycline and chloramphenicol. The number of mutants was no higher than that found in the normal strain of *E. coli*.

The action of the Mu character in strain 635 thus seems to be limited to the Sm resistance locus.

Attempts to locate the Mu character on the chromosome. In the Treffers strain the mutant has been found to be a gene located near the threonine locus. The mutator factor in *S. typhimurium* LT7, described by Miyake (1960), was located by means of sexual recombination and was found to lie between the methionine and the threonine loci.

This part of the investigation was started with a series of recombinations with the Treffers strain, to provide control material concerning the behavior of a known mutator factor in conjugation experiments. The results (Table 2) concur with the assumption that the mutator factor in the Treffers strain is a gene, since it segregates among the genetic markers in the expected way. The data may also be taken to indicate that the factor is located in the threonine region, between methionine and threonine, probably quite close to the threonine locus. A more precise localization, however, has been considered as beyond the scope of the present investigation. The results obtained agree with those of Miyake (1960) but not with those of Skaar (1956), as far as the localization of the mutator gene is concerned.

Attention was next turned to the new mutator strain. In crosses with K-12 Hfr strains, strain 635 acted as an F− recipient. The recombination frequencies, expressed as per cent of the Hfr population, were lower than in crosses of K-12 Hfr with K-12 F−, more similar to that found in crosses between K-12 Hfr and B (Gorini and Gundersen, 1961). From the experimental series no. 2 in Table 2, it may be seen that the segregation of the two biochemical markers employed agree with the generally accepted hypothesis. A small discrepancy between these results and those found in crossing Hfr Cavalli with K-12 M+(meth−thre−) may be due either to the fact that the methionine markers are different in the two recipient strains, or to the comparatively low number of recombinants analyzed in the first series of recombinations. The behavior of the mutator character in strain 635, on the other hand, differs significantly from that of the mutator gene in the Treffers strain.

On the whole, the results of the experiments with the strain Hfr Cavalli, reported in Table 2, are not very conclusive with regard to the mutator factor. Thus, it was decided to try another donor in conjugations with strain 635. Some results obtained with strain Hfr Hayes are accordingly included in Table 2. These experiments support the previous impression that the

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**Table 1. Reversion rates of some auxotrophic mutants of *Escherichia coli* 635**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Selection</th>
<th>No. of cells in inoculum</th>
<th>No. of tubes with growth</th>
<th>Mutation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>thre−hi−Mu+</td>
<td>thre-independent</td>
<td>8.6 x 10^6</td>
<td>29</td>
<td>6.95 x 10^-10</td>
</tr>
<tr>
<td>hi−Mu+</td>
<td>hi-independent</td>
<td>1.5 x 10^6</td>
<td>1</td>
<td>9.51 x 10^-12</td>
</tr>
<tr>
<td>thre−ilva−Mu+</td>
<td>ilva-independent</td>
<td>5.0 x 10^6</td>
<td>3</td>
<td>8.26 x 10^-11</td>
</tr>
<tr>
<td>thre−meth−Mu+</td>
<td>meth-independent</td>
<td>1.0 x 10^6</td>
<td>4</td>
<td>5.78 x 10^-11</td>
</tr>
<tr>
<td>thre−Mu−</td>
<td>thre-independent</td>
<td>1.2 x 10^6</td>
<td>20</td>
<td>2.95 x 10^-10</td>
</tr>
</tbody>
</table>
Table 2. Conjugation with Hfr donors and two types of strains with mutator factors as recipients

<table>
<thead>
<tr>
<th>Expt. series</th>
<th>Conjugation system</th>
<th>Selective system</th>
<th>Segregation of unselected markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor</td>
<td>Recipient</td>
<td>Markers</td>
</tr>
<tr>
<td>1</td>
<td>Hfr Cavalli*</td>
<td>K-12 1 M</td>
<td>tryp+thre+</td>
</tr>
<tr>
<td></td>
<td>tryp-</td>
<td>thre^-meth-</td>
<td>tryp^-meth+</td>
</tr>
<tr>
<td>2</td>
<td>Hfr Cavalli</td>
<td>635 Mu+</td>
<td>tryp^-meth+</td>
</tr>
<tr>
<td></td>
<td>tryp-</td>
<td>thre^-meth-</td>
<td>tryp^-meth+</td>
</tr>
<tr>
<td>3</td>
<td>Hfr Cavalli</td>
<td>635 Mu+</td>
<td>tryp^hi+</td>
</tr>
<tr>
<td></td>
<td>tryp-</td>
<td>thre^-hi-</td>
<td>tryp^-thre+</td>
</tr>
<tr>
<td>4</td>
<td>Hfr Cavalli</td>
<td>635 Mu+</td>
<td>tryp^-ilva+</td>
</tr>
<tr>
<td></td>
<td>tryp-</td>
<td>thre^-ilva-</td>
<td>tryp^-ilva+</td>
</tr>
<tr>
<td>5</td>
<td>Hfr Hayes†</td>
<td>635 Mu+</td>
<td>meth^-thre+</td>
</tr>
<tr>
<td></td>
<td>meth^-B;</td>
<td>thre^-hi-</td>
<td></td>
</tr>
</tbody>
</table>

* Hfr Cavalli injects markers into the K-12 recipient in the order: T6, lac, leu, thre, meth.
† Hfr Hayes injects markers into the K-12 recipient in the order: Thre, leu, lac, T6, gal, hi.
‡ This number of colonies scored is too low to justify a calculation in per cent.

biochemical markers follow the mode of transfer of chromosomal factors, but the new mutator factor does not.

In conjugations between strains 635 and K-12 Hfrs, the loss of the mutator factor was in no instance significantly higher than the spontaneous loss of this character, regardless of which markers were used for the selection of recombinants. The character does certainly not segregate among the recombinants in the way one would expect from any gene located on the bacterial chromosome. Also, one would not expect to find such a high spontaneous loss of a gene as that found for this character.

The results thus seem to indicate that the factor is located not on the chromosome but in the cytoplasm. The genetic nature of the factor may then be similar to that of the F factor or the colicinogen (Jacob, Schaeffer, and Wollman, 1960).

Infertility of the Mu character in strain 635. Since the spontaneous loss of the Mu character has been found to be fairly high, one would expect the efficiency of infection to be very low for an episome-like character. Otherwise the Mu-negative cells would be reinfected immediately from the Mu+ cells in the population. It thus seemed necessary to render a 635 Mu+ strain F+ by infection in order to enhance a possible transmission of the mutator factor. This has been necessary in the transmission of certain colicinogens (Fredericq, 1957).

The strain 635 Mu+thre^-hi^- was accordingly made F+ by infection from W6 F^-meth^- at first, some experiments were performed to transfer the Mu character from a 635 Mu+ to a 635 Mu-. The mutants 635 Mu+F+thre^-hi^- and 635 Mu^-F^-leu^- were grown to the exponential phase in complete media, and then mixed in equal proportions in fresh, complete medium. This culture was incubated for 20 hr without shaking at 37 C. Since there is no way of selecting for the mutator character directly, the culture was plated out in dilutions on minimal-leucine agar plates to give single colonies of the recipient strain. Of 361 colonies isolated in three separate experiments, 7 (2%) colonies had become mutators. These 7 colonies were still leu- but Mu+. Former experiments had shown that a spontaneous reversion of a Mu- strain to Mu+ does not occur. The mutator character thus seems to have the properties of a transmissible cytoplasmic factor.

Attempts were made to transfer the Mu character into a K-12 strain. During these assays the same strain was used as the donor of the Mu character, and K-12 T 71 was employed as a recipient. The latter strain is a prototroph, Sm+, val+, Mu-, and sensitive to the colicin-like substance produced by strain 635 and to the phage plk0. Of 239 colonies isolated on minimal agar as previously described, 7 colonies (3%) had become mutators. These colonies had all other characteristics of K-12.
With 635 Mu+ F- thre- hi- as the donor and K-12 T 71 as a recipient, an experiment was carried out to investigate the necessity of the F factor for the transmission of the Mu character. Approximately 1,600 colonies were scored by replication. Of these colonies, three were found to be Mu+. These colonies were found to be E. coli K-12. The transmission frequency in this case was approximately 0.2%. Thus, the F factor definitely enhances the frequency of transmission; its presence, however, is not indispensable.

It is important to know whether the mutator is still infectious in an organism which has been made resistant to Sm. It is also of great interest to know which property would be transmitted in this case, the mutator factor or Sm resistance (Watanabe and Fukisawa, 1961a). To elucidate these problems, three mixed-culture experiments were carried out with the system 635 Mu+F- thre- hi- and 635 Mu-F Sm+leu- . Of a total of 789 colonies scored, 7 colonies (1%) with the other markers of the recipient strain were found to be mutants. These colonies were mutators and not streptomycin resistant.

Elimination of the Mu factor in strain 635 with acriflavine. Hirota and Iijima (1957) found that acriflavine was an effective agent for the elimination of the F factor in E. coli. Watanabe and Fukisawa (1961b) similarly reported the elimination of their episomal multiple resistance factor by acridine dyes and by the combination of UV irradiation and acridine treatment. To confirm our conclusion that the mutator factor is a factor comparable to the previously described episomes, treatment with acriflavine was tried by the technique described by Hirota and Iijima (1957). The cells were grown in complete medium to the exponential phase, and then inoculated into fresh complete medium with and without 20 µg of acriflavine per ml. These cultures were incubated overnight without shaking at 37 C. The cells were then centrifuged, resuspended in saline, diluted, and spread to give single colonies on complete plates. Of 895 colonies isolated after treatment with acriflavine in three separate experiments, 178 (20%) had lost the mutator character. Of 704 colonies isolated in the control experiments without acriflavine, 34 (4.8%) had lost the mutator character.

In one experimental series, the cells to be treated with acriflavine were previously irradiated with UV. The cells were grown to the exponential phase, and 5 ml of the culture was irradiated for 105 sec by a 15-w Sterilectric UV lamp at a distance of 40 cm from the sample. Then 5 ml of fresh medium was added, and the culture was incubated for 1 hr at 37 C, whereupon it was treated with acriflavine as previously described. Of 216 colonies isolated after UV irradiation plus acriflavine treatment, 160 (74%) had lost their mutator character. Of 341 colonies isolated after UV irradiation only, 72 colonies (21%) had lost their mutator character.

These results show that the Mu character in strain 635, with regard to its behavior to acridine dyes and UV, behaves in the manner previously described for autonomous, episomal factors.

Effect of UV irradiation of the donor on the efficiency of transfer of the Mu factor in strain 635. Since Watanabe and Fukisawa (1961a) reported that UV irradiation of the donor cell before the actual transfer increased the frequency of transmission of the resistance factor 10 to 100 times, this was also tried in the present case. The donor cell, 635 Mu+F+ thre- hi-, was irradiated 1 hr before the mixed-culture experiment. Of 745 colonies isolated after conjugation of a culture of the irradiated donor with 635 Mu-F leu-, none had received the mutator character. Of 635 colonies isolated in the control experiment with the unirradiated donor, 10 (1.5%) had received the mutator character.

Thus, in the case of the Mu character of strain 635, UV irradiation of the donor before conjugation impaired the transmission instead of enhancing it. This, of course, may be due to a high sensitivity of the Mu factor to UV irradiation, a sensitivity which makes the cells lose the factor before the cell to cell contact is established.

DISCUSSION

The present investigation indicates that the mechanism of the enhanced mutation rate to Sm resistance in E. coli 635 is different from the types earlier described (Treffers et al., 1954; Goldstein and Smoot, 1955; Jyssum, 1960; Miyake, 1960). The action of the mutator character in strain 635 seems to be strictly limited to the Sm locus, whereas the mutator gene in the Treffers type of mutator acts on a number of different loci around the chromosome, including that of Sm resistance. The type of mutator action described by Jyssum (1960) also
seems to include other loci than that of Sm resistance. A mutator gene affecting one single gene locus was found in maize (McClintock, 1951).

The mutator of *S. typhimurium* LT7 is of the Treffers type, with increased mutation frequency in either direction; i.e., the mutation frequency to auxotrophy is increased, and the auxotrophs back-mutate at a much higher rate than is usual in a strain not possessing the mutator gene. According to Kirchner (1960), the ability of the LT7 M+ strains (95% of those tested) to respond to base analogues shows that this gene, in some way, facilitates an event leading to a transition. He suggested that the LT7 mutator gene controls the production or overproduction of some compound(s) responsible for this behavior. This explanation does not agree entirely with the fact that a few loci do not seem to be affected by the mutator gene in the Treffers strain. The mutants used in these cases, however, might represent subloci similar to the hi- gene of Labrum (1953), and the mutagen-stable mutants described by Demerec (1953).

The mechanism of action of the mutator factor in strain 635 is not understood. It will be of interest to see whether this mutator action affects the whole Sm locus, or only, as preliminary experiments have indicated, the resistance and not the dependence.

Not only the range of action, but also the location of the mutator character, is different in strain 635, compared with the Treffers strain. Whereas the mutator character in the Treffers strain of *E. coli* and in *S. typhimurium* LT7 has been found to be a gene, which can be mapped by means of transduction or sexual recombination, no such mapping has been successful in the case of strain 635. This fact, together with the fact that the mutator factor of strain 635 is lost with a fairly high frequency (approximately \(10^{-2}\)) suggests that this mutator character is of cytoplasmic nature.

The extrachromosomal nature of the mutator character in strain 635 is further supported by the fact that it has been possible to transmit the mutator factor by infection from a 635 Mu+ to a 635 Mu−, and also to a K-12 Mu− strain. This may be explained as an episome-mediated transfer. The transfer is enhanced by the presence of the F factor in the donor organism. It has been possible to increase the frequency of elimination of the mutator factor from strain 635 by treatment with acriflavine and by UV irradiation, features which have also been described for other genetic determinants of an episomal nature, such as the F factor (Hirota and Iijima, 1957).

Whether the mutator factor is actually an episome of the nature of deoxyribonucleic acid remains to be determined. Its behavior, however, shows great similarity to that of the resistance factor of Watanabe and Fukissawa (1961a), although its mode of action is entirely different.

**LITERATURE CITED**


