NEW TYPE OF STREPTOMYCIN RESISTANCE RESULTING FROM ACTION OF THE EPISOMELIKE MUTATOR FACTOR IN *ESCHERICHIA COLI*

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ABSTRACT

GUNDERSEN, WENCHE B. (Oslo University, Oslo, Norway). New type of streptomycin resistance resulting from action of the episomelike mutator factor in *Escherichia coli*. J. Bacteriol. **86**:510–516. 1963.—Analyses have been performed to elucidate the genetic nature of the streptomycin resistance that results from the action of the previously described episomelike mutator factor in *Escherichia coli*. This streptomycin resistance has been found to differ from ordinary one-step streptomycin resistance. The new type of streptomycin resistance, "mutator resistance," can be lost, either spontaneously or by treatment with ultraviolet light and acriflavine. It is more stable in a K-12 strain than in the original *E. coli* strain 635. Mutator resistance segregates like a chromosomal marker in genetic crosses, and is located near the ordinary streptomycin locus. The locus for mutator resistance is distinct from that of ordinary streptomycin resistance, apparently located further toward the threonine region. Mutator resistance, unlike ordinary one-step streptomycin resistance, appears as a dominant character. The possibilities of its being a suppressor or regulator mutation are discussed.

Discovery of a new type of genetic instability with episome-mediated transfer in *Escherichia coli* was the subject of a previous communication from this laboratory (Gundersen, Jyssum, and Lie, 1962). The genetic determinant for this instability was not found to follow the pattern of a chromosomal marker in genetic crosses. It was found to possess some properties generally ascribed to genetic determinants of episome nature: (i) a spontaneous loss of the character, enhanced by treatment with ultraviolet (UV) irradiation and acridine dyes; (ii) a loss which apparently is permanent; and (iii) a transfer to a mutator-negative cell by infection, favored by the presence of the F factor in the donor. The cells possessing the genetic determinant for this instability are characterized by an enhanced mutant frequency (of the order of 10^{-4}) to streptomycin resistance, whereas no other loci seem to be affected. Resistance to streptomycin resulting from the action of this genetic determinant seems to be of the streptomycin-indifference type (Watanabe and Watanabe, 1959).

It has been the purpose of this investigation to search for an explanation of how the mutator factor works to confer streptomycin resistance on the cell, and to find whether the resulting resistance is genetically identical with ordinary streptomycin resistance as it is described in the literature (Newcombe and Nyholm, 1950; Demerec et al., 1949; Lederberg, 1951; Hashimoto, 1960).

MATERIALS AND METHODS

The methodology and experimental manipulations used in this investigation were analogous to those previously described (Gundersen et al., 1962).

Bacterial strains. E. coli strain 635 was isolated from a patient; *E. coli* K-12 1M and K-12 T71 were obtained from H. P. Treffers; Hfr Cavalli and Hfr Hayes were received from L. Gorini; W6 F^+ was supplied by Ida Örskov.

RESULTS

Stability of streptomycin resistance in strain 635Mu+. The mutator factor (*Mu*) of strain 635 may be thought to exert its effect on streptomycin resistance of the cell in various ways. The most obvious hypothesis is that the extrachromosomal particle would, in fact, occasionally be incorporated into the chromosome by a copy-choice mechanism during replication, thus producing a permanent streptomycin-resistant (*str-r*) cell. Since streptomycin sensitivity is a

dominant character with regard to streptomycin resistance (Lederberg, 1951), the cell could well possess several determinants for streptomycin resistance, and still be phenotypically streptomycin sensitive (*str-s*). This situation might correspond to that of an episome-infected cell with several autonomously replicating particles. Due to structural similarities with the genetic determinant for streptomycin sensitivity, the episomal unit might be expected to be attracted to this site of the chromosome, somewhat like the "memorizing" F particle, F'-*pro* and F'-*lac* (Jacob and Adelberg, 1959). This event might facilitate a copy-choice mechanism, resulting in a streptomycin-resistant cell after elimination of the streptomycin-sensitive allele.

According to this hypothesis, the resulting streptomycin-resistant cell should have all the characteristics of the ordinary streptomycin-indifferent cell.

Since the ordinary type of streptomycin resistance is known to be a very stable, inheritable character, stability studies were first undertaken. Some data from such investigations have been recorded in Table 1.

TABLE 1. *Stability of the streptomycin character in 635Mu⁺ and 635Mu⁻* *

Strain	Treatment			No. of colonies scored	No. of *str-s* colonies	Loss of *str-r*
	UV irradiation		Acriflavine			
	Time	Distance				
	sec	*cm*	*μg/ml*			%
635*Mu*⁺ *str-r*				1010	9	0.9
	60	20		961	26	2.7
	120	20		781	81	10.3
	105	40	20	605	90	14.8
			20	120	5	4.1
635*Mu*⁻ *str-r*	105	40	20	331	0	<0.3

* Strains 635*Mu*⁺*str-r* and 635*Mu*⁻*str-r* were grown to the logarithmic phase in complete broth; 5 ml of the culture were poured into a petri dish and irradiated by UV light. The irradiated cultures were then grown in complete broth overnight with or without acriflavine. Dilutions were plated out to ensure single colonies, which were scored for their streptomycin character.

The results indicate a significant instability in streptomycin resistance obtained after action of the mutator factor. This is in striking contrast to the stability of the ordinary type of streptomycin indifference of the same strain.

As the next step in the investigation, colonies of strain 635*Mu*⁺*str-r* which grew after treatment with UV irradiation and acriflavine were also checked for their *Mu* character. All the colonies that had lost streptomycin resistance by this treatment had also lost the *Mu* character. Colonies (25) which were still streptomycin resistant after this treatment were investigated, and they were all found to carry the mutator factor in the infectious form.

The stability studies thus seem to provide strong evidence against the hypothesis presented.

The mutator factor is obviously not permanently incorporated in the bacterial chromosome by a copy-choice mechanism. It is still possible, however, that the mutator factor is attached to the chromosome, since it has been reported that episomes attached to the chromosome can be lost either spontaneously or by treatment with UV irradiation (Sneath, 1962).

When the stability of streptomycin resistance is compared with that of the mutator character after similar treatment of strain 635, a significant difference is found (*see* Gundersen et al., 1962). Thus, it seems that the factor in this strain has in some ways achieved a greater stability after development of streptomycin resistance. It may be suggested that this is caused by an attachment to the chromosome.

The stability studies, furthermore, strongly indicated that the normal allele for streptomycin sensitivity must still be present in cells which are phenotypically streptomycin resistant. Thus, resistance caused by action of the mutator factor would seem to be a dominant character.

Properties of the Mu factor in a K-12 strain. From previous studies, it is known that the mutator factor infects K-12 strains (Gundersen et al., 1962). Since it is more convenient to perform genetic analyses in the elaborate K-12 system, it appeared natural to proceed with genetic studies in such a system.

At first, experiments were performed to ascertain that behavior of the *Mu* factor in strain K-12 is similar to that in strain 635. In Table 2 some experiments have been recorded which show that strain K-12 T71*Mu*⁺ may be used as an F⁻ strain in crosses with K-12 Hfr strains. The crosses performed with the double mutant

TABLE 2. *Elucidation of extrachromosomal nature of Mu factor in a K-12 strain*

Conjugation system		Selective system		Segregation of unselected markers		
Donor	Recipient	Marker	No. scored*	Marker	Received	
					No.	Per cent
K10*try*†	T71*Mu*⁺*thr his* #1, #2, and #3	*try*⁺*thr*⁺	686	*his*⁺	11	1.6
		try⁺*his*⁺	686	*thr*⁺	352	51.3
3000B₁*met*‡	T71*Mu*⁺*thr his* #1, #2, and #3	*met*⁺*thr*⁺	590	*his*⁺	5	0.8
		met⁺*his*⁺	590	*thr*⁺	243	41.2

* None were *Mu*⁻.
† Hfr Cavalli.
‡ Hfr Hayes.

TABLE 3. *Stability of Mu factor and streptomycin character in K-12 T71Mu*⁺

Strain	UV irradiation* for	No. of colonies scored	No. of colonies losing *Mu* or *str-r*	Per cent loss
	sec			
T71*Mu*⁺	—†	336	0	<0.3
T71*Mu*⁺*thr*	60†	379	1	0.26
his	60	96	0	<1
	105	96	0	<1
T71*Mu*⁺*thr*	60	208	0	<0.5
str-r	90	210	0	<0.5
T71*Mu*⁺*thr* *his str-r*	90	96	0	<1
T71*arg*‡*str-r*	60, 75, 90	593	0	<0.2

* Distance from UV light source was 20 cm in all experiments; 20 μg of acriflavine per ml were added in all experiments, except where otherwise indicated.
† Acriflavine omitted.
‡ Arginine marker.

T71*Mu*⁺ *thr his* (*thr*, threonine; *his*, histidine) as recipient, and either K10*try* (tryptophan); Hfr Cavalli) or 3000B₁*met* (methionine; Hfr Hayes) as donor indicated that the mutator factor behaved as an extrachromosomal factor in a K-12 strain as well as in the original strain 635. This indication was further supported by the fact that K-12 T71*Mu*⁺, the recipient strain, was able to donate the *Mu* factor to a mutator-negative cell by infection.

The results seem to indicate that spontaneous loss of the *Mu* factor in K-12 T71*Mu*⁺ is significantly less than in strain 635, where spontaneous loss of the *Mu* factor was around 4% (Gundersen et al., 1962). In Table 3, several experiments have been recorded which were performed to elucidate this problem. Evidently, the *Mu* factor, as well as the streptomycin resistance caused by the action of the factor, has a greater stability in strain K-12 T71 than in the original strain 635. According to the experiments performed, behavior of the *Mu* factor is the same in strain K-12 T71 as in strain 635 with regard to its extrachromosomal localization as well as its potential modification of the streptomycin sensitivity of the cell. Genetic studies of the resulting streptomycin resistance may thus be performed in the K-12 system, taking advantage of its well-established genetic system as well as of its greater stability.

Chromosomal localization of streptomycin resistance resulting from action of the mutator factor. If streptomycin resistance caused by action of the *Mu* factor, the "mutator resistance," is the result of some modification of the bacterial chromosome, it should behave in bacterial crosses as a regular chromosomal factor.

Accordingly, mutator resistance was the subject of conventional genetic mapping, using various mutants of K-12 T71*Mu*⁺ as well as different Hfr donors. The data presented in Table 4 indicate that streptomycin resistance inflicted by the *Mu* factor segregates like a chromosomal marker. This marker seems to be located between threonine and histidine, clockwise from histidine, just like the ordinary marker determining streptomycin sensitivity and resistance in *E. coli* K-12. It should be noted as an important feature of the system that the colonies which were streptomycin sensitive among the progeny in these crosses were still *Mu*⁺.

TABLE 4. *Segregation of streptomycin character in bacterial crosses*

Conjugation system		Selective system				Segregation of unselected markers		
Donor	Recipient	Marker	No. of colonies scored	*Str-s* character		Marker	Received	
				No.	Per cent		No.	Per cent
K10*try*	T71*Mu*$^+$*thr his str-r**	*try*$^+$*thr*$^+$	1072	199	18.5	*his*$^+$	5	0.46
		try$^+$*his*$^+$	1209	334	27.6	*thr*$^+$	590	48.8
K10*try*	T71*Mu*$^+$*thr ser*†*str-r*	*try*$^+$*thr*$^+$	480	49	10.2	*ser*$^+$	39	8.1
		try$^+$*ser*$^+$	480	134	27.9	*thr*$^+$	213	44.3
3000B$_1$*met*	T71*Mu*$^+$*thr his str-r*	*met*$^+$*thr*$^+$	480	0	<0.2	*his*$^+$	5	1.0
		met$^+$*his*$^+$	480	0	<0.2	*thr*$^+$	268	55.8
K10*try*	T71*Mu*$^-$*thr his str-r*‡	*try*$^+$*thr*$^+$	960	44	4.6	*his*$^+$	5	0.5
		try$^+$*his*$^+$	888	282	31.7	*thr*$^+$	383	43.1

* Four different *thr his* mutants.
† Serine marker.
‡ Two different *thr his* mutants.

The chromosomal site of mutator resistance was also compared with that of ordinary streptomycin resistance in the corresponding strain K-12 T71 carrying the same biochemical markers. From the results presented in Table 4 it is seen that, whereas the distribution of the biochemical markers in the two comparable crosses is similar within the error of this somewhat crude system, the distribution of the streptomycin markers shows some difference. When threonine was used as a selective marker, 18.5% of the progeny received the marker for streptomycin sensitivity upon the use of T71*Mu*$^+$ (mutator resistant) as the recipient, whereas only 4.6% of the progeny received this marker when the regular T71 (streptomycin resistant) was used as a recipient. With use of histidine as a selective marker, the distribution was 27.6% in the case of T71*Mu*$^+$ (mutator resistant), and 31.7% in the case of the regular T71 (streptomycin resistant). These differences, if significant, suggest that we are dealing with two different loci. It looks as though the genetic determinant for mutator resistance is located closer to the threonine locus in Hfr Cavalli than the regular marker for streptomycin resistance.

Test for nonidentity of mutator resistance and ordinary streptomycin resistance. Mutator resistance certainly behaves as a chromosomal marker in K-12 crosses. Since the conventional mapping may be taken to indicate a certain genetic distance between mutator resistance and ordinary streptomycin resistance, the hypothesis was adopted that we are, indeed, dealing with two different loci. If genetic determinants for the two types of streptomycin resistance are allelic, or if they are located in adjacent loci on the bacterial chromosome, one would not expect to find streptomycin-sensitive colonies among the progeny from crosses of two strains that are both streptomycin-resistant. In Table 5, several crosses between streptomycin-resistant K-12 strains have been recorded. The donor in these experiments was a streptomycin-resistant mutant, K10 *try str-r*, which is a one-step mutant of the streptomycin-indifferent type. Recipients were various streptomycin-resistant mutants from the strains used as acceptors in the crosses recorded in Table 4. In accordance with our predictions, no streptomycin-sensitive colonies were found among the progeny of any two strains with ordinary streptomycin resistance. When a donor strain with the ordinary kind of streptomycin resistance, however, was crossed with an acceptor strain carrying mutator resistance, a remarkable number of streptomycin-sensitive clones appeared among the progeny. The results, thus, are in agreement with the hypothesis indicating the existence of two distinct streptomycin-resistance loci. Since mutator resistance apparently also differs from ordinary streptomycin resistance by being a dominant character, it might be tentatively characterized as a suppressor locus.

Test for identity of ordinary streptomycin re-

TABLE 5. *Conjugation among various types of streptomycin-resistant strains*

Conjugation system		Selective system				Segregation of unselected markers		
Donor	Recipient	Marker	No. of colonies scored	*Str-s* character		Marker	Received	
				No.	Per cent		No.	Per cent
K10*try str-r*	T71*Mu⁻thr his str-r**	*try⁺thr⁺*	840	0	<0.1	*his⁺*	46	5.4
		try⁺his⁺	840	0	<0.1	*thr⁺*	429	51.0
K10*try str-r*	T71*Mu⁺thr his str-r*†	*try⁺thr⁺*	1467	148	10.8	*his⁺*	13	0.9
		try⁺his⁺	1440	79	5.4	*thr⁺*	720	50.0

* Two different *thr his* mutants.
† Four different *thr his* mutants.

TABLE 6. *Conjugations using another type of mutator strain as recipient*

Conjugation system		Selective system*			Segregation of unselected markers		
Donor	Recipient	Marker	*Str-s* character		Marker	Received	
			No.	Per cent		No.	Per Cent
K10*try*	Treffers *thr his str-r*	*try⁺thr⁺*	15	6.2	*his⁺*	7	2.9
		try⁺his⁺	99	41.2	*thr⁺*	132	55.0
K10*try str-r*	Treffers *thr his str-r*	*try⁺thr⁺*	0	<0.4	*his⁺*	16	6.6
		try⁺thr⁺	0	<0.4	*thr⁺*	168	70.0

* Number of colonies scored in each cross was 240.

sistance and resistance caused by the mutator allele of Treffers. The mutator allele, originally detected in *E. coli* K-12 by Treffers, Spinelli, and Belser (1954), increases the mutation rate in several independent loci, including that of streptomycin resistance. Strain K-12 1M, carrying this allele, has previously been used in our investigation for comparative purposes (Gundersen et al., 1962). The mutator allele is apparently similar to the one in *Salmonella typhimurium* strain LT7 described by Miyake (1960). According to Kirchner (1960), action of this mutator allele may be due to the production of some compound(s) leading to a transition. Therefore, one might expect streptomycin resistance caused by this type of mutator to be identical to ordinary one-step streptomycin indifference.

Consequently, streptomycin resistance caused by the Treffers mutator allele was tested in a genetic cross against the regular type of streptomycin resistance in the system previously described. The data from such mating experiments (Table 6) are in accordance with our predictions. The mutator allele in the Treffers strain thus seems to affect the streptomycin locus directly. This is in agreement with the theory put forward by Kirchner. The results (Table 6) further serve to underline the difference between the two types of mutator strains.

DISCUSSION

The present studies concerning streptomycin resistance caused by action of the mutator factor originally discovered in *E. coli* 635 indicate that it is genetically different from ordinary, one-step streptomycin resistance of the streptomycin-indifference type.

The cells with resistance caused by the extrachromosomal particle, the mutator resistance, can convert to sensitivity spontaneously, after treatment with UV light alone, or with a combination of UV light and acriflavine. This loss seems to be permanent. The cells, which in this way become streptomycin-sensitive, simultane-

ously lose their mutator factor. The criterion for calling an extrachromosomal particle an episome is its dual nature. Either it can replicate as an autonomous particle, or it can be attached to the chromosome. These two situations are usually mutually exclusive. Such is apparently the case with the F particle as well as with temperate bacteriophages (Jacob and Wollman, 1961). If, in the case of the present mutator factor, the cell becomes streptomycin-resistant as a result of an attachment of one of the particles to the chromosome, the cell must be assumed to possess the particle *also* in the autonomous state, or at least in an infectious form, since the mutator-resistant cell is still infectious. The result of such an infection is acquisition of the mutator property, not the resistance (*see* Gundersen et al., 1962). That episomic elements, when fixed to the chromosome, are sometimes lost, especially after treatment with UV light, is known (Sneath, 1962). When in the present system the cell returns to streptomycin sensitivity, either spontaneously or after treatment, the mutator factor does not return to the autonomous state, but is permanently lost. The fact that the mutator-resistant cell can revert so easily to streptomycin sensitivity, in sharp contrast to the ordinary one-step streptomycin-resistant cell, apparently reflects a fundamental difference in the two types of resistance. It may be suggested that the streptomycin-sensitive allele is present all the time in the mutator-resistant cell. This, of course, requires that mutator resistance be a dominant character, since the cell is phenotypically streptomycin-resistant even when the gene for streptomycin sensitivity is present in the cell all the time. Such a dominance is in sharp contrast to ordinary one-step streptomycin resistance, which is a recessive character (Lederberg, 1951). Streptomycin resistance introduced into a cell by the Resistance Transfer Factor of Watanabe and Fukasawa (1961*a*, *b*) must also be a dominant character, since cells are phenotypically streptomycin-resistant from the moment the RTF factor enters the cell. In these cells, the determinant for streptomycin sensitivity is also present all the time. When the RTF factor is removed from the cell by treatment with acridine dyes, the cell again becomes streptomycin-sensitive.

The mutator resistance studied in the present paper definitely behaves as a chromosomal factor. In crosses K-12 × K-12, the new type of streptomycin resistance segregates as a character located on the chromosome near the methionine region. This may correspond well with the ordinary marker determining streptomycin resistance. When, however, mutator resistance is further compared with a regular streptomycin-resistance marker, there is some indication that the former is located closer to the threonine locus (in Hfr Cavalli) than the latter.

Recombination experiments between an ordinary streptomycin-resistant mutant of Hfr Cavalli and mutator-resistant K-12 cells gave a high number of streptomycin-sensitive clones among the progeny. This feature strongly supports the hypothesis that the two kinds of resistance represent separate loci with a certain distance between them. The results may be taken to indicate that the locus determining mutator resistance is indeed localized somewhere between the ordinary gene for streptomycin resistance and the threonine region.

It is tempting to look for an explanation of the findings concerning the genetics of streptomycin resistance in the current concept of gene regulation as described by Jacob and Monod (1961). However, since as yet too little is known about the biochemistry of the streptomycin system, it seems reasonable at the present stage to confine the discussion to the use of more conventional genetic terms. Two distinct types of mutations have previously been described which affect the sensitivity to streptomycin in *E. coli*. We have the ordinary one-step mutation to streptomycin indifference. In this case, the streptomycin-sensitive allele is dominant to the streptomycin-insensitive allele. Mutations of this type all appear to occur in the same locus. On the other hand, we have mutation to streptomycin dependence. Mutations to streptomycin resistance and to streptomycin dependence have been shown to be closely linked nonidentical alleles (Hashimoto, 1960). Furthermore, a back-mutation from dependence to nondependence has been reported not to take place at the original site, but to be the result of a second mutation in a suppressor locus which is closely linked. It may be suggested that the mutator resistance described in the present paper is the result of an attachment of the mutator particle at the site of a suppressor locus, resulting in interference with the expression of the ordinary streptomycin locus. It might be of interest to find out whether the site of mutator

resistance is the same as that of the back-mutation from streptomycin dependence to nondependence. This seems unlikely, however, since the genetic distance between mutator resistance and regular streptomycin resistance seems to be too great to permit cotransduction such as demonstrated by Hashimoto.

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