Functional Analysis of Second-step Host Specificity Mutations in Unstable *Escherichia coli* Heterozygotes

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SUMMARY

From an *Escherichia coli* strain K12 carrying a temperature-sensitive host-specific modification (*hsm*) mutation, second-step mutants have been isolated that are completely deficient in modification and restriction. Complementation analysis has revealed that one group of these mutants is impaired in the specificity gene *hss*, while in the other group of mutant strains both mutations, i.e. the first-step temperature-sensitive and the second-step which impairs restriction and modification completely, are located in *hsm*. Analysis of the heterozygotes used in the complementation experiments suggested a *cis*- and tandem arrangement of the *hs* and *leu* genes in haploid, segregating exconjugants; however, the attachment of these genes to a cryptic plasmid was not excluded.

INTRODUCTION

To study the mode of interaction of the polypeptides it is necessary to establish in which of the three genes involved in restriction and modification, *hss*, *hsr* and *hsm* (Glover & Colson, 1969; Arber & Linn, 1969), any isolated mutation is located. Based upon a complementation analysis, genotypes for mutants temperature-sensitive (ts) in restriction and modification have been proposed (Hubáček & Glover, 1970). Second-step mutants isolated from these temperature-sensitive mutants were deficient in both restriction and modification. They were of two types: the first type contains mutations which appear to be located in the *hss* gene, the second type contains mutations located in the *hsm* gene. A crucial genetical test to support the idea that for the expression of restriction the interaction of *hss*, *hsr* and *hsm* gene products is required, is to demonstrate complementation for temperature-sensitive restriction and modification between these two types of mutant. This paper reports the results of such a test.

METHODS

Bacteria. *Escherichia coli* strains used are described in Table 1.

Bacteriophages. λ vir, phage P1 and F2 male-specific phage.

Media. Bacterial and phage buffers, M9 medium, L-broth, L-amino acid supplements, thiamin and streptomycin concentrations used were as described previously (Hubáček & Glover, 1970). M9 medium and L-broth were solidified with 1.5% (w/v) Oxoid agar no. 3. For soft agar overlays 0.6% nutrient agar or water-soft agar was used.

Tests for restriction and modification, transduction procedure, preparation of F' factors and methods for construction of F' merodiploids. These were described previously (Glover, 1970; Hubáček & Glover, 1970). A *Thr*⁻ derivative of HfrH was transduced with P1 grown on *Escherichia coli* with a mutation in the *hs* gene; from among the *thr*⁺ transductants

Table 1. *Escherichia coli* strains employed in the experiments

Strain	Host specificity phenotypes	Other characteristics	Source or reference
HfrH	$r_K^+ m_K^+$	<i>thi str-s</i>	Hayes, 1953
HfrH-1	$r_K^+ m_K^+$	<i>thr thi str-s</i>	<i>thr</i> mutation introduced by NTG
HfrH-5K	$r_K^- m_K^+$	<i>thi str-s</i>	5K or VIII mutations were transduced by P1 to HfrH-1
HfrH-VIII	$r_K^- m_K^-$	<i>thi str-s</i>	Willetts & Clark, 1969
Hfr JC5088	—	<i>ilv thr spe-r str-s rec A56</i>	Appleyard, 1954
C ₆₀₀	$r_K^+ m_K^+$	<i>thr leu thi lac str-s</i>	Glover, 1970
C ₆₀₀ -4K	$r_K^- m_K^-$	<i>thr leu serB thi str-r</i> (λ) ⁺	Hubáček & Glover, 1970
C ₆₀₀ -5K	$r_K^- m_K^+$	<i>thr leu thi str-r</i>	Arber & Dussoix, 1962
B251	$r_B^+ m_B^+$	<i>try str-s</i>	Bertani & Weiglé, 1953
C	$r^- m^-$	<i>serB str-s</i>	ts mutation 1-30 transduced by P1 to 4K (Hubáček & Glover, 1970)
4K-29	$r_{ts}^+ m_{ts}^+$	<i>leu thi str-r</i> (λ) ⁺	
IV	$r^- m^-$	<i>leu thi str-t</i> (λ) ⁺	By EMS mutagenesis of 4K-29 (Hubáček & Glover, 1970)
VIII	$r^- m^-$	<i>leu thi str-r</i> (λ) ⁺	
X	$r^- m^-$	<i>leu thi str-r</i> (λ) ⁺	
XI	$r^- m^-$	<i>leu thi str-r</i> (λ) ⁺	
IV-28	$r^- m^-$	<i>leu thi thy str-r</i> (λ) ⁺	By NTG mutagenesis
IV-33	$r^- m^-$	<i>leu thi thy str-r</i> (λ) ⁺	By NTG mutagenesis
AB1157	$r_K^+ m_K^+$	<i>thr leu arg pro his</i>	Howard-Flanders & Theriot, 1966
AB2463	$r_K^+ m_K^+$	<i>thi str-r</i>	
		<i>thr leu arg pro his</i>	
		<i>thi rec A13 str-r</i>	
F'X 8011	$r_K^- m_K^-$	<i>F' lac⁺/lac Δ U169 str-s</i>	Hubáček & Glover, 1970
F'KLF1	$F'r_K^+ m_K^+ / r_K^+ m_K^+$	<i>F'thr⁺ leu⁺/AB 2463</i>	The construction of the diploids according to Low's technique (Low, 1968)
F'VIII	$F'r_K^- m_K^- / r_K^+ m_K^+$	<i>F'thr⁺ leu⁺/AB 2463</i>	
F'5K	$F'r_K^- m_K^+ / r_K^+ m_K^+$	<i>F'thr⁺ leu⁺/AB 2463</i>	
F'B 2.1	$F'r_B^- m_B^- / r_K^+ m_K^+$	<i>F'thr⁺ leu⁺/AB 2463</i>	

—, Host specificity phenotype not tested.

HfrH $r_K^- m_K^-$ (VIII) and $r_K^- m_K^+$ (5K) colonies were selected. The partial diploids were constructed by crossing HfrH strains with AB2463 *recA* recipient according to the method described by Low (1968). These merodiploids were used as donors in crosses with appropriate host specificity mutants. The strains were grown in supplemented M9 medium, mated, and *Leu*⁺ colonies (selected on M9 medium) were purified and their sensitivity to f2 phage, segregation of *Leu*⁺/*Leu*⁻ and *m*⁺/*m*⁻, and complementation for restriction and modification were followed.

Nitrosoguanidine (NTG) mutagenesis was carried out following the procedure of Adelberg, Mandel & Chein Ching Chen (1965).

RESULTS

All the temperature-sensitive mutants described by Hubáček & Glover (1970) were found to have mutations in the *hsm* gene. Second-step mutants that had completely lost the capacity to modify at 30 °C were isolated from strain 4K-29, carrying mutation *tsr-30*. These were analysed in complementation experiments with the F-prime F'B2.1 $r_B^- m_B^+$ and formed two groups. Mutant strains VIII and X appeared to have both *hssK* and *hssR* intact because the diploids expressed both K-specific and B-specific restriction and modification, implying that the second mutation in both parent strains lay in the *hsm* gene. IV and XI, however, showed only B-specific restriction and modification, suggesting that in these

Table 2. Restriction and modification phenotype of the diploids

Expt no.	Mutant no.	Temperature (°C)	Haploid phenotype		Restriction*			Modification†		Phenotype of merodiploid‡
			Recipient	F- prime	λ_p .K	λ_p .B	λ_p .C	K	B	
1	5K	30	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.004	0.01	0.7	0.0001	$r_K^- m_K^+$
		40	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.009	0.02	0.6	0.00003	$r_K^- m_K^+$
2	4K-29	30	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.002	0.01	0.5	0.0001	$r_K^- m_K^+$
		40	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.15	1.0	0.009	0.0004	$r_K^- m_K^+$
3	X	30	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	0.5	—	0.5	0.001	—	$r_K^- m_K^-$
		40	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	0.5	—	0.3	0.0004	—	$r_K^- m_K^-$
4	XI	30	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	0.3	—	0.3	0.1	—	$r_K^- m_K^+$
		40	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	0.3	—	0.3	0.002	—	$r_K^- m_K^+$
5	IV	30	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.2	1.0	0.1	0.000001	$r_K^- m_K^+$
		40	$r_K^- m_K^+$	$r_K^- m_K^-$ (VIII)	1.0	0.15	1.0	0.0002	0.000003	$r_K^- m_K^+$
6	IV	30	$r_K^- m_K^+$	$r_B^- m_B^+$	0.003	0.1	0.002	0.003	0.7	$r_B^- m_B^+$
		40	$r_K^- m_K^+$	$r_B^- m_B^+$	0.01	0.2	0.002	0.0002	0.8	$r_B^- m_B^+$

* Restriction indicates the e.o.p. of λ_{vir} . K, B and C on the diploids.† Modification indicates the e.o.p. of phage produced by the merodiploids on *Escherichia coli* K, B and C indicator strains.‡ The sensitivity to f_2 , segregation of leu^+/leu^- and complementation were used to check the F status of the diploids.
—, Not tested.

strains the second mutation was in the *hssK* gene and that the *hsr* gene was functional because the restriction function missing in the F-prime was efficiently restored (Expt 6, Table 2).

If the interpretation of these experiments is correct it should be possible to demonstrate complementation between these two types of second-step mutants. An F-prime strain VIII was constructed and used to test for complementation with a number of host specificity mutants. The results of the complementation tests are presented in Table 2. The first three experiments are controls. With 5K $r_K^- m_K^+$ (*hssK*⁺*hsr*⁻*hsm*⁺), complementation for K-specific restriction was observed (Expt 1). If the second mutation in strain VIII is not *trans*-dominant one would expect temperature-sensitive restriction and modification in the diploid constructed between F'VIII and 4K-29 $r_{ts}^+ m_{ts}^+$ recipient and this was actually found (Expt 2). The diploid F'VIII/F-X is $r^- m^-$ and no complementation for the restriction and modification was observed as expected for the two second-step mutants of the same genotype (Expt 3).

In the diploids constructed between F'VIII and second-step mutant strains IV and XI (Expts 4 and 5) the positive complementation of temperature-sensitive modification confirms the proposed *hs* genotype of the mutants. However, the level of the modification at 30 °C is low (only 10 % of λ in the burst is modified) and no complementation for restriction was detectable in the diploids.

Because instability in the diploids may play a role in the observed inefficient complementation, the *recA* mutation was introduced into mutant IV. The donor JC5088 strain with proximal *thy*⁺ and *recA56* genes was mated with IV-33 *thy* recipient. A *thy*⁺, UV^s colony (IV-33-5) was isolated which showed about 1000-fold lower efficiency of integration when mated with HfrH. The *recA* mutation does not show any marked effect on cell growth in M9 medium; but only about 7 % of cells in the culture form colonies after plating on LA medium. The effect on growth and viability of the cell population was even more pronounced for merodiploids constructed between F'5K, F'VIII and F'KLF1 and the recipient IV-33-5 *recA*. The cultures not only produce a lower fraction of colony-forming bacteria on LA, but in M9 medium growth is reduced; as an example, see the F'5K/IV-33-5 diploid in Table 3. As a result of this poor growth the strains did not plate λ efficiently on LA and M9 agar plates.

Because of these difficulties in using *recA* derivatives to analyse complementation for temperature-sensitive restriction and modification, the screening technique for isolation of partial diploids was modified. The filamentous growth of the diploids at 40 °C and the effect of *recA* mutation on their viability suggested that the cells infected with an F' proximal merogenote might be at a selective disadvantage towards F⁻ cells in the culture. To avoid this problem, in further complementation analysis one could attempt to use purified *leu*⁺ exconjugants which are f2 resistant but of the *m*⁺ phenotype at 30 °C. In such bacteria the *hs-leu* segment is postulated to be either a part of a cryptic plasmid or duplicated in *cis* and tandem to the original one. Of special interest in this case are *cis* heterozygous duplications. As shown in Table 4, the expected f2⁺ and *m*⁺ phenotype was found among the *leu*⁺ exconjugants made in this way only with IV-28 *thy*⁻ recipient (100 colonies tested); with IV and IV-28 *thy*⁺ spontaneous revertants, among 10 colonies tested only f2-sensitive recombinants were detected. The frequency of *leu*⁺ colonies in the cross F'VIII $\times$ IV-28 *thy*⁻ is nearly 20-fold lower than in the two other crosses and the f2r, *m*⁺ phenotype among them appears dependent upon *thy* mutation.

To be sure that these recombinants are completely F⁻ in character, the cultures were mated with ABI157 on M9 agar with supplements to select *Leu* recombinants (counter-

Table 3. The growth properties of *Escherichia coli* strains

Strain	Temperature of cultivation (°C)	No. of colonies/ml* on		C.f.u.† on LA (%)
		LA	M9A	
IV-33	30	3.4×10^8	4.0×10^8	85.0
IV-33-5	30	3.2×10^7	4.6×10^8	6.9
F'5K/IV-33-5	30	1.6×10^5	5.5×10^7	0.2
F'5K/IV-33-5	37	2.5×10^5	1.1×10^8	0.2

* No. of colony-forming units after overnight cultivation in M9 medium with appropriate supplements.

† Fraction of cells in the population able to form colonies.

Table 4. The frequency of f_2^+ , m^+ phenotypes among leu^+ colonies in F'VIII $\times$ IV or IV-28 thy^- and thy^+ crosses

F' donor strain and the recipients (all strains of r^-m^- phenotype) were grown in supplemented M9 at 37 °C; thymine concentration 40 µg/ml. Equal 0.1 ml volumes of the recipient and serial 10-fold dilutions of the donor were mixed and plated on supplemented M9 to select leu^+ colonies. After purification the sensitivity to f_2 phage and the modification phenotype were tested.

Recipient	Frequency of leu^+ colonies*	No. of leu^+ colonies tested	No. of phenotypes			
			f_2^s, m^+	f_2^s, m^-	f_2^r, m^+	f_2^r, m^-
IV	81.4	10	7	3	0	0
IV-28 thy^-	5.0	100	7	1	60	32
IV-28 thy^+	87.8	10	9	1	0	0

* No. of colonies/100 donor cells.

Table 5. Restriction and modification in *Escherichia coli* segregating heterozygotes

The cultures were grown overnight in M9 with thiamine and thymine at the appropriate temperatures and then tested for restriction and modification as described previously (Hubáček & Glover, 1970).

Phenotype no.	Growth temperature (°C)	Restriction			Modification (K)
		$\lambda_v.K$	$\lambda_v.B$	$\lambda_v.C$	
IV-28 thy^- -5	30	0.5	0.004	0.01	0.7
	40	1.0	0.3	1.0	0.02
IV-28 thy^- -1	30	0.7	0.007	0.01	0.5
	40	1.0	0.06	0.4	0.05

selection against the donor was on agar without thymine) and no such colonies were isolated. In the cross with the Hfr strain JC5088, which transfers *thy* as a proximal marker, about 10 % of thy^+ recombinants per donor were obtained (counter-selection against donor was with streptomycin). The surface exclusion index, measured as the ratio of thy^+ *str-r* progeny in 60 min 1:1 crosses with JC5088 as the donor and F⁻ strains IV-28 and the strain in question, was about 2. The leu^+ , f_2^+ recombinants were also mated with an F-*lac* donor, strain 8011, and lac^+ thy^- *str-r* colonies obtained; a result which again implies that the exconjugants behave as F⁻.

Complementation for restriction and modification in two such F⁻ recombinants is shown in Table 5. It is evident that this type of recombinant can express complementation of temperature-sensitive restriction and modification of the two second-step mutants VIII and IV.

The F⁻, f_2^+ exconjugants which show complementation were grown in Penassay broth,

Fig. 1. The interrupted mating experiment. The donor IV-28-9, sensitive to nalidixic acid, was grown overnight in M9 with lactose, thiamine and thymine and the recipient, IV, resistant to 20 $\mu\text{g/ml}$ nalidixic acid grown overnight in M9 with glucose, thiamine and leucine were precultivated in Penassay broth 120 min. After 1:10 mixing the bacteria were filtered on Millipore membrane filters; the filters were placed on preheated LA and exposed at 37 °C. At intervals the bacteria were resuspended and the pairs interrupted by agitating in a Vortex mixer. Suspensions were diluted and plated on M9 agar with lactose, thiamine, leucine and nalidixic acid to select *lac*⁺ colonies (Δ) and M9 agar with glucose, thiamine and nalidixic acid to select *leu*⁺ colonies ($\circ$).

and after dilution the bacteria were plated on M9 with thiamine, leucine and thymine. After replication on to M9 agar without leucine and LA agar seeded with *Escherichia coli* κ and C soft-agar overlays, the number of *leu*⁻ and *m*⁻ segregants was counted. These two markers segregated independently; about 20 % *leu*⁻ and 10 % *m*⁻ colonies were found under these experimental conditions. The phenotype of the F⁻, f2^r exconjugant was found to be an unstable Leu⁺ which segregates with the same frequency to Leu⁻ as does stable Leu⁺. Acriflavine increased the frequency of Leu⁻ colonies up to 60 %. To interpret this effect, the sensitivity of the F⁻, f2^r *leu*⁺ colonies and *leu*⁻ segregants to acriflavine (5 $\mu\text{g/ml}$) was tested. [¹⁴C]Uracil incorporation into RNA was measured according to Nakamura & Suganuma (1972). Uracil incorporation was less sensitive to acriflavine in *leu*⁻ segregants than in *leu*⁺ heterozygotes.

The segregating exconjugants were infected with an F-*lac*⁺ episome; about 80 % of *lac*⁺ colonies tested were found sensitive to f2 phage. One *lac*⁺, f2^r colony (IV-28-9) was mated with strain IV as a recipient on M9 agar without thymine to select against the donor, and *lac*⁺ and *leu*⁺ recombinants were selected. Seventy % of *lac*⁺ and 3 % of *leu*⁺ recombinants/100 donor cells were obtained. The purified recombinants were tested for unselected markers; more than 90 % of *lac*⁺ colonies were found to be *leu*⁻, while 60 % of *leu*⁺ colonies were also *lac*⁺. Only the *lac*⁺ recombinants were sensitive to f2 phage.

An interrupted mating experiment was performed on membrane filters because of the low frequency of *leu*⁺ colonies after a 1000-fold dilution of the mating mixture in Penassay broth. As shown in Fig. 1, *lac*⁺ recombinants appeared at about 10 min and *leu*⁺ recombinants at about 20 min after mixing the donor and recipient bacteria.

DISCUSSION

Mutant VIII appears to carry wild-type *hssK* because the merodiploid with $F'r_B^-m_B^+$ expresses K-specific restriction and modification (Hubáček & Glover, 1970); it has wild-type *hsr* since it is able to restore by complementation the restriction deficiency in $r_B^-m_B^+$ and $r_K^-m_K^+$ recipients (Table 2; Expt 1). Both mutations, first-step and second-step, are placed in *hsm* and we can write the genotype of this mutant as $hssK^+hsr^+hsm^-$.

On the other hand, mutant IV does not express K-specific restriction and modification in diploids with $F'r_B^-m_B^+$ (Table 2; Expt 6), but it appears to have normal wild-type *hsr* because it complements the restriction deficiencies in $r_B^-m_B^+$ and $r_K^-m_K^+$ mutant strains. We suggest that the genotype of the mutant IV is $hssK^-hsr^+hsm_a^+$.

From the host specificity genotypes one can expect complementation to produce temperature-sensitive restriction and modification in diploids constructed between F' VIII and mutant IV. The difficulties in the detection of the complementation, especially for restriction, in the diploids were not overcome even in *recA* derivatives of the strain. The filamentous growth, specifically of the diploid cells at 40 °C, and the lower viability of *recA* cells infected with F' element, would suggest some selective disadvantage of the F' cells in the bacterial population. The construction of a heterozygous duplication with a tandem arrangement of *hs* genes was thought to be a way to solve the problem. One might expect such exconjugants to show complementation, to be F^- but to segregate *hs* and *leu* markers. This type of recombinant was isolated at relatively high frequency using a *thy^-* recipient strain. The role of this mutation in this process as well as the origin of such exconjugants is not clear. Certain mechanisms of prezygotic or postzygotic elimination of the plasmid genes are operating only with the proximal F merogenote transferred into the recipient strain pre-cultivated in M9 medium.

The exconjugants were found to be completely F^- in character (active as a genetic recipient; lack of activity as a genetic donor; low surface-exclusion index; ability to be infected with F ; resistance to male-specific phage), with apparent complementation for restriction and modification; they segregate *hs* and *leu* markers. After reinfection with $F-lac$ the diploid behaves like a secondary F' strain transferring *lac* with high frequency as well as *leu* which was originally located on the proximal F merogenote.

Alternatively, one can assume *leu* and *hs* to be attached to a cryptic plasmid; the effect of acriflavine on the segregation of the *leu* marker is compatible with this interpretation. However, the higher sensitivity of RNA synthesis to acriflavine in the diploids weakens this argument. The *leu^-* segregants could grow faster under these conditions and thus predominate in the population. It is possible that two genetically different variants of the same plasmid can exist more or less stably in the same cell despite the incompatibility barrier which normally prevents this situation.

Positive results in complementation of temperature-sensitive restriction and modification has revealed that the genotypes of the two groups of mutants deficient in both restriction and modification are as we have proposed (Hubáček & Glover, 1970). One mutant is impaired in the specificity gene while the other is mutated in the *hsm* gene, and both have an intact *hsr* gene. It is evident that a mutation in *hsm* can express the same phenotypic effect on restriction and modification as the impaired *hss* gene and that for restriction the interaction of *hss*, *hsr* and *hsm* gene products is required. Studies of the sub-unit structure of the restriction endonuclease from *Escherichia coli* B by Eskin & Linn (1972) are entirely consistent with this model.

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REFERENCES

- ADELBERG, E. A., MANDEL, M. & CHEIN CHING CHEN, G. (1965). Optimal conditions for mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K-12. *Biochemical and Biophysical Research Communications* **18**, 788–795.
- APPLEYARD, R. K. (1954). Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K-12. *Genetics* **39**, 440–452.
- ARBER, W. & DUSSOIX, D. (1962). Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ . *Journal of Molecular Biology* **5**, 18–36.
- ARBER, W. & LINN, S. (1969). DNA modification and restriction. *Annual Reviews of Biochemistry* **38**, 467–500.
- BERTANI, G. & WEIGLÉ, J. J. (1953). Host controlled variation in bacterial viruses. *Journal of Bacteriology* **65**, 113–121.
- ESKIN, B. & LINN, S. (1972). The deoxyribonucleic acid modification and restriction enzymes of *Escherichia coli* B. II. Purification, subunit structure, and catalytic properties of the restriction endonuclease, *Journal of Biological Chemistry* **247**, 6183–6191.
- GLOVER, S. W. (1970). Functional analysis of host-specificity mutants in *Escherichia coli*. *Genetical Research* **15**, 237–250.
- GLOVER, S. W. & COLSON, C. (1969). Genetics of host-controlled restriction and modification in *Escherichia coli*. *Genetical Research* **13**, 227–240.
- HAYES, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spring Harbor Symposium Quantitative Biology* **18**, 75–93.
- HOWARD-FLANDERS, P. & THERIOT, L. (1966). Mutations of *Escherichia coli* K 12 defective in DNA repair and genetic recombination. *Genetics* **53**, 1137–1150.
- HUBÁČEK, J. & GLOVER, S. W. (1970). Complementation analysis of temperature-sensitive host specificity mutations in *Escherichia coli*. *Journal of Molecular Biology* **50**, 111–127.
- LOW, B. (1968). Formation of merodiploids in matings with a class of *rec*[−] recipient strains of *Escherichia coli* K 12. *Proceedings of the National Academy of Sciences of the United States of America* **60**, 160–167.
- NAKAMURA, H. & SUGANUMA, A. (1972). Membrane mutation associated with sensitivity to acriflavine in *Escherichia coli*. *Journal of Bacteriology* **110**, 329–335.
- WILLETTS, N. S. & CLARK, A. J. (1969). Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. *Journal of Bacteriology* **100**, 231–239.