

EXPERIMENTAL GENETICS

EFFECT OF DIFFERENCES IN THE *rec*-GENOTYPE OF *Escherichia coli* AS A FUNCTION OF F' SEX FACTOR

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Functions of the F' sex factor have been shown to depend not only on injury to the genes controlling the recombining ability of bacterial cells, but also on suppression of mutations of *rec* genes. Suppression of mutations of these genes is accompanied by ability of the sex factor to mobilize the chromosome for transfer from *rec*⁻ cells. It is postulated that reversions of the *rec A* gene also lead to restoration of the possibility of recombination between F' sex factor and the bacterial chromosome.

KEY WORDS: *rec*-genotype; suppression; reversions.

Recent work has shown that about 10 genes participate in determining the ability of *Escherichia coli* to recombine [2]. Since the identification of these genes has been carried out by isolating the appropriate mutants, the presence of these mutants can be used to investigate the effect differences in the *rec*⁻ genotype not only on recombination accompanying conjugation of the bacteria, but also on the type of recombination that determines the mechanism of mobilization of the chromosome for transfer of the F' sex factor [5].

The object of the present investigation was to study the effect of mutations of individual *rec* genes and their suppression in *E. coli* cells on the function of the sex factor.

EXPERIMENTAL METHOD

As the F' sex factor the following variants of this factor were used: APF' 27 - Pro⁺ Leu⁺ Thr⁺ and APP' 30 - Pro⁺ Leu⁺ Thr⁺,* identified by the writer previously [1] and later transferred to J 62 F⁻ - pro his try str^r. Sex factors were introduced into AB 2463 *rec A*⁻, Jc 5519 *rec B21*⁻ *rec C22*⁻, Jc 8679 *rec B21*⁻ *rec C22*⁻ sbs A23⁻, and Jc 9604 *rec A56*⁻ *rec B21*⁻ *rec C22*⁻ sbs A23⁻ cells, and also into Jc 9604-252 and Jc 9604-131 cells, originating from Jc 9604 cells but behaving as Rec⁺ cells in their phenotype as a result of hypothetical suppression of the *rec A* gene in Jc 9604-252 or reversion of the *rec A* gene in 9604-131. To analyze the functions of the sex factors, cells of the above-mentioned strains were crossed by standard methods with PA-373 F⁻ - thr leu his arg met nal^r str^r recipient cells. The mixtures, conjugating at 37°C, were taken out after 60 min on selective media, so that the frequency of transfer of the F' factors and the frequency of transfer of chromosome markers by them could be determined. The behavior of the sex factors was studied in control experiments after their introduction into AB 1157 cells.

EXPERIMENTAL RESULTS

After introduction of the sex factors from *E. coli* J 62 F⁻ into cells of strains differing in *rec*-genotype, one clone was selected from each crossing. In this way 14 clonal cultures (including the control), differing in their *rec*-genotype and having one of the sex factors studied, were selected.

*The nomenclature of Demerec et al. [3] is used in this paper and the abbreviations of the symbols for the genetic markers are taken from Taylor and Trotter [6].

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TABLE 1. Transfer of Sex Factors F' - Pro⁺ Leu⁺ Thr⁺ and Chromosome Markers by Donor Cells with Different Powers of Recombination in Crosses with PA-373 F⁻ Recipient Cells

Strains of cells carrying F' sex factor; their genotypes and phenotypes	F' sex factor of donor cells	Frequency of genetic transfer (per donor cell)		
		episomal markers		chromosomal markers
		Thr ⁺	Leu ⁺	Met ⁺
AB2463 thr leu pro hisarg rec A ⁻	APF'30—			
Phenotype — Rec ⁻	Pro ⁺ Leu ⁺ Thr ⁺	4.10 ⁻⁴	4,8 10 ⁻⁴	0
	APF'27—			
Jc 5519 thr leu pro his arg rec	Pro ⁺ Leu ⁺ Thr ⁺	2,7 10 ⁻⁵	4,9 10 ⁻⁵	0
B21 ⁻ rec C22 ⁻ . Phenotype — Rec ⁻	APF'30	3,8 10 ⁻⁴	5,3 10 ⁻⁴	0,17 10 ⁻⁷
Jc 8679 thr leu pro his arg rec	APE'27	5,5 10 ⁻⁴	6,9 10 ⁻⁴	0
B21 ⁻ rec C22 ⁻	APF'30	6,7 10 ⁻³	7,1 10 ⁻³	0,46 10 ⁻⁷
C22 ⁻ sbc A23. Phenotype — Rec ⁺				
Jc 9604 thr leu pro his arg rec	APF'27	7,4 10 ⁻³	10 10 ⁻³	0,12 10 ⁻⁷
A56 ⁻ rec				0
B21 ⁻ rec C22 ⁻ sbc A23 ⁻ . Phenotype — Rec ⁻	APF'30	0,04 10 ⁻⁴	9,1 10 ⁻⁴	0
Jc 9604—252 thr leu pro his arg	APF'27	3,3 10 ⁻⁵	5,6 10 ⁻⁵	1,4 10 ⁻⁷
rec A56 ⁻ rec				
B21 ⁻ rec C22 ⁻ sbc A23 ⁻ . Phenotype — Rec ⁺	APF'30	1,7 10 ⁻³	1,9 10 ⁻³	0,035 10 ⁻⁷
Jc 9604 —131 thr leu pro his arg	APF'27	5,1 10 ⁻⁴	6,1 10 ⁻⁴	
rec A56 ⁻ rec				
B21 ⁻ rec C22 ⁻ sbc A23 ⁻ . Phenotype — Rec ⁺	APF'30	3,1 10 ⁻³	3,6 10 ⁻³	1,2 10 ⁻⁷
AB1157 thr leu pro his arg rec ⁺	APF'27	4,07 10 ⁻⁴	5,5 10 ⁻⁴	0
	APF'30	1,10 ⁻²	1,01 10 ⁻²	1,4 10 ⁻⁷
	APF'27	2,9 10 ⁻³	3,2 10 ⁻³	0,12 10 ⁻⁷

To determine the ability of the sex factors to undergo transfer from cells with lesions of different rec genes, Thr⁺Str^r and Leu⁺Str^r neurodiploids were selected from crosses of all clonal cultures with PA-373. To determine the ability of the sex factor to mobilize the transfer of chromosomal markers, Met⁺Str^r recombinants were selected from these crosses. The results are given in Table 1.

As Table 1 shows, cells of strains AB 2463 and Jc 9604 transfer the sex factors studied in these experiments to recipient cells, but less frequently than cells of the other strains. They do not transfer chromosomal markers, however,

Cells of strains Jc 5519 transfer sex factors with a frequency intermediate between the frequencies characteristic of AB 2463, Jc 9604, and AB 1157 (control) cells.

Cells of strain Jc 8679, defective for the rec B21 and rec C22 genes, but phenotypically behaving as Rec⁺ cells because of suppression of these genes, carry out both episomal and chromosomal transfer with a frequency approximately the same as that observed in cells with no defects in their rec genes (AB 1157).

Cells of strains Jc 9604-252 and Jc 9604-131, in which suppression of the rec A⁻ gene or reversion of this gene (respectively) is postulated, and which behave phenotypically as Rec⁺ cells, are also capable of transfer of both sex factor and Met⁺ chromosomal marker, and of doing so at a frequency close to the frequency of transfer by AB 1157 cells.

The results indicate that the functions of F' sex factor depend not only on injuries to the genes controlling the recombination power of bacterial cells [4], but also on suppression of mutations of the rec genes. Suppression of mutations of these genes is accompanied by ability of the sex factor to mobilize the chromosome for transfer from rec⁻ cells. If it can be shown that the cells of strain Jc 9604-131 do in fact carry reversions for the rec A gene, the results will signify that reversions of the rec A gene can also lead to restoration of ability to recombine between the F' sex factor and the bacterial chromosome.

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