EXPERIMENTAL GENETICS

CHROMOSOMAL TRANSFER OF F-MERODIPLOIDS OF RECOMBINATION-DEFECTIVE MUTANTS OF *Escherichia coli* K-12

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F-merodiploids arising from rec⁻ mutants carry out chromosomal transfer at a lower frequency than F-merodiploids from the rec⁺ strain. The degree of this decrease differs significantly in merodiploids arising from different rec⁻ mutants.

The results of investigation of the mechanisms of chromosomal transfer by F-merodiploids of *Escherichia coli* suggested that mobilization of the F'–lac⁺ sex factor of the chromosome for transfer takes place as the result of a crossover between these two structures [6-9]. If, therefore, the hypothesis regarding the role of genetic exchange in chromosomal transfer is correct, the frequency of transfer from donor cells carrying sex factor F' to recipient cells must reflect the effectiveness of recombination between sex factor and chromosome. As Clowes and Moody [3] showed, this hypothesis can be tested by determining the donor ability of F-merodiploids carrying the rec⁻ allele [2].

This paper describes the results of experiments to study the chromosomal transfer ability of F-merodiploids obtained by introducing different F'–lac⁺ factors into cells of three recombination-defective mutants of *E. coli* K-12 of independent origin.

EXPERIMENTAL METHOD

Mutants rec⁻ 48, rec⁻ 15, and rec⁻ 75, isolated from *E. coli* P-678 F⁻ Thr⁻ Leu⁻ B_1 lac⁻ T_1^R T_6^R Str^R were used in the investigation. Cells of the rec⁻ mutants [1], and also cells of the original rec⁺ strain (control) were infected with sex factor F'–lac, originating from cells of intermediate donors *E. coli* 200 PS, 1485, and W-1485. The sex factor was introduced by mixing donor cells and recipient cells in the ratio of 1 : 2 in nutrient broth and then by keeping the mixture for 1 h at 37°. Seedings were then taken from these cells on Endo's agar with 250 units/ml streptomycin, and after incubation of the cultures, lactose-positive colonies were picked off, and clonal cultures of cells with chromosomal markers of the recipient strain were obtained from them. The presence of sex factor in these clonal cultures was confirmed by determining their sensitivity to specific "male" phage f_2. The donor ability of the isolated merodiploids rec⁻ lac⁻/F'–lac⁺ and rec⁺ lac⁻/F'–lac⁺ (control) was determined in crossings carried out by standard methods [4] with cells of recipient strain *E. coli* J-62 F⁻ Pro⁻ Try⁻ His⁻ lac⁻ T_6^S Str^r. For this purpose, Pro⁺ recombinants were selected and their frequency determined.

EXPERIMENTAL RESULTS

Cells of all rec⁻ and rec⁺ strains were infected with each of the three sex factors F'–lac⁺, three clonal cultures being selected from each in order to study chromosomal transfer.

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TABLE 1. Frequency of Chromosomal Transfer Effected by rec⁻ F-merodiploids

rec⁻ and rec⁺ donors		Frequency of appearance of Pro+ recombinants					
F' infecting factors, their origin	rec⁻ mutants infected with F' factors	per 100 donor cells	rec⁻/rec⁺ index, %	per 100 donor cells	rec⁻/rec⁺ index, %	per 100 donor cells	rec⁻/rec⁺ index, %
		1 clone		2 clones		3 clones	
(E. coli 200PS lac⁻)	rec⁻48	0,004	0,15	0,004	0,05	0,001	0,04
	rec⁻15	0,32	11,4	0,5	6,3	2,46	16,4
	rec⁻75	1,5	54,0	1,8	22,5	1,4	50,0
	rec⁺	2,8	(100)	8,0	(100)	2,8	(100)
F'—lac⁺ (E. coli 1485 lac⁻)	rec⁻48	0,013	0,43	0,001	0,01	0,001	0,05
	rec⁻15	0,43	14,3	1,2	15,0	0,34	17,0
	rec⁻75	1,4	46,7	2,1	26,2	1,1	55,0
	rec⁺	3,0	(100)	8,0	(100)	2,0	(100)
F'—lac⁺ (E. coli W-1485 lac⁻)	rec⁻48	0,005	1,2	0,0003	0,04	0,001	0,3
	rec⁻15	0,19	44,2	0,26	34,7	0,14	46,7
	rec⁻74	1,5	350	2,2	300	0,24	83,3
	rec⁺	0,43	(100)	0,75	(100)	0,3	(100)

Note. The number of Pro⁺ recombinants was calculated as a percentage of the number of the same recombinants formed in crosses with cells of rec⁺ F-merodiploids (taken as a 100%).

The frequency of appearance of Pro⁺ recombinants in crosses between cells of F-merdiploids rec⁻ and rec⁺ of all clonal cultures with J-62 cells is shown in Table 1. The frequency of Pro⁺ recombinants in the crosses in which F-merodiploids arising from cells of the rec⁻ 48 mutant, and carrying sex factor F'—lac⁺ from the donor strain 200 PS, were used as donors was 0.04-0.15% of the frequency of the same recombinants in the control crosses. The frequency of recombinants in crosses in which F-merodiploids obtained from mutants rec⁻ 15 and rec⁻ 75, carrying sex factor F'—lac⁺ also from 200 PS, were the donors was 6.3-16.4 and 22-54%, respectively, of the frequency observed in the control crossing.

It is also clear from Table 1 that rec⁻ F-merodiploids arising from the mutant rec⁻ 48 and carrying F'—lac⁺ factor from strain 1485 give rise to Pro⁺ recombinants also with a low frequency, only 0.01-0.05% of the frequency in the control crossing. In crosses between F-merodiploids obtained from rec⁻ 15 and rec⁻ 75 mutants and E. coli F⁻ J-62, the frequency of recombinants was 14-17 and 26-55% of the frequency in the control.

Finally, in crosses of F-merodiploids arising from the rec⁻ 48 mutant and carrying sex factor F'—lac⁺ from cells of strain W-1485, with E. coli J-62 the frequency of recombinants was 0.04-1.2% of the control frequency, whereas F-merodiploids of mutant rec⁻ 15 were able to bring about the formation of 35-47% of the possible number of recombinants. The effectiveness of the F-merodiploids of mutant rec⁻ 75 was greater, because in the crosses in which they were used as donors, the frequency of appearance of the Pro⁺ recombinants was almost normal or was greater than in the control crosses.

It must be pointed out that clonal differences were found in individual crosses.

The frequency of appearance of Pro⁺ recombinants in most crosses in which rec⁻ F-merodiploids were used as donors was thus lower than that observed in the control crossing in which the donors were rec⁺ F-merodiploids. In connection with existing data indicating that mutations of the rec genes have no significant effect on the formation of contacts between cells during conjugation or on transfer of the F'—sex factor [3, 5], the reduced frequency of Pro⁺ recombinants observed following crossing of the studied rec⁻ F-merodiploids can be explained by disturbance of the mobilization of the chromosome for transfer. Differences in the frequency of transfer observed in merodiploids arising from different rec⁻ mutants evidently reflect differences in rec⁻ mutations in strains rec⁻ 48, rec⁻ 15, and rec⁻ 75. This explanation agrees with the assumption made by Wilkins [10], who studied the effect of rec⁻ mutations of a different origin on the chromosomal transfer by F'—lac⁺ sex factor. The results of the present investigation thus confirm the hypothesis that chromosomal transfer takes place as a result of recombination between sex factor and chromosome.

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