DEFECTIVE MUTANTS OF SEX FACTOR IN E. coli K-12 CELLS

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Sex factor (F factor) in donor cells of Escherichia coli K-12 consists of a double helix of DNA with a length of 200-400 Å, or about 2% of the total length of the bacterial chromosome [2, 3, 6, 7]. It is postulated that this structure must carry about 100 different genes [4], which can be identified by isolation of the corresponding mutants. After Hirota and Iijima [5] had described mutation of F factor present in F^+ cells, Cuzin [1] isolated clones of cells carrying a mutant sex factor F^1 (F-genota). Since the isolated cells were characterized by lowered ability to transmit F-genota and chromosome genes, this type of mutant F^1 was called defective.

The object of the present investigation was to seek defective mutants of F factor in cells of cultures of E. coli Hfr H, where it is integrated in the chromosome.

EXPERIMENTAL METHOD

The donor strain used in the investigation was E. coli Hfr $HB_1^-S^S$, the cells of which transmit genes during conjugation in the order O-T-L-Try-Pro-His . . . F. The recipient strains were E. coli P678F$^-$T$^-$L$^-$B$_1^-$Lac$^-$S^r and E. coli J62 F$-$Pro$^-$Try$^-$His$^-$Lac$^-$S^r.*

Eight hour broth cultures of the donor strain were irradiated on a type RUM-7 x-ray apparatus in a dose giving 10^{-6} of surviving cells. Samples were taken after irradiation and seeded on meat-peptone agar in dishes to obtain isolated colonies. After 24 h, to determine their phenotype, the colonies were transferred by means of a replica technique to minimal media, to meat-peptone agar (MPA) with streptomycin, and to MPA covered with a suspension of phage F2, to which the donor cells are sensitive, and to select $T^+L^+S^+$, Try^+S^r, Pro^+S^r, and His^+S^r recombinants, they were transferred to selective media (minimal agar, glucose, streptomycin, thiamine, or essential amino acids), covered by previously washed cells of one of the recipient strains. Colonies which gave no growth after 48 h on any of the selective media were subcultured from the original dishes into meat-peptone broth, incubated for 8 h, and then mixed with 8 h broth cultures of recipient strains (ratio 1:10). The mixtures of crossed cells were incubated at 37° for 2 h, after which their dilutions were seeded on selective media as described above to select recombinants.

TABLE 1. Frequency of Recombinants in Crosses of Clone Cultures of E. coli Hfr H with E. coli P678F and J62F$^-$

Cross	Sensitivity of donor to phage F2	Frequency of recombinants (in %; per 100 donor cells)			
		$T^+\alpha^+S^r$	Try^+S^r	Pro^+S^r	His^+S^r
HfrH × P678F$^-$	+	2,88			
HfrH × J62F$^-$	+		0,72	0,22	0,023
232-P × P678F$^-$	+	0			
232-P × J62F$^-$	+		0	0	0
18-P × P678F$^-$	+	0			
18-P × J62F$^-$	+		0	0	0
14-P × P678F$^-$	+	0,0001			
14-P × J62F$^-$	+		0,00045		
4-P × P678F$^-$	+	0,000028			
4-P × J62F$^-$	+		0,000121		

Colonies obtained after seeding samples from unirradiated donor cultures were investigated as controls.

*The following abbreviations are used in the paper: B_1 thiamine, T threonine, L leucine, Try tryptophan, Pro proline, His histidine, Lac^+/Lac^- ability/inability to ferment lactose, S^S/S^r sensitivity/resistance to streptomycin, O leading edge of transferred segment of chromosome.

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TABLE 2. Crosses of E. coli Hfr C and 1485 (F-lac) with Clone Cultures 232-P and 18-P

Cross	Frequency of recombinants $B_1^+ S^S$
HfrC × 232-P	$3{,}8 \cdot 10^{-3}$
HfrC × 18-P	$8{,}2 \cdot 10^{-3}$
1485 (F—lac) × 232-P	$2{,}3 \cdot 10^{-2}$
1485 (F—lac) × 18-P	$2{,}3 \cdot 10^{-2}$

EXPERIMENTAL RESULTS

After x-ray irradiation of cultures of the donor strain 6500 isolated colonies were investigated. Their phenotype corresponded to the phenotype of colonies developing from cells not irradiated with x-rays and possessing normal donor ability (control). Tests of irradiated cells for donor ability by transferring colonies to agar covered with recipient cells showed that 23 colonies gave no growth on the various selective media. After clone cultures had been obtained from these colonies, each was crossed with cultures of recipient strains. It was found that the donor ability of the cells had been modified in only four clone cultures, conventionally designated 232-P, 18-P, 14-P, and 4-P.

Data illustrating the ability of the cells of the clone cultures to donate genes are given in Table 1.

It is clear from Table 1 that, while remaining sensitive to specific phage F2, the cells of the isolated clone cultures differed in their donor ability.

Cells of clones 14-P and 4-P donate genes with exceptionally low frequency. Since the frequency of recombination in crosses when the donors were cells of clone cultures 14-P and 4-P was comparable with the frequency of recombination usually observed with the use of donor cultures of F^+ type, to discover the state (integrated or autonomous) of the sex factor in such cells from crosses 14-P × P678F$^-$, 14-P × J62F$^-$, 4-P × P678F$^-$, and 4-P × J62F$^-$, 30 of each of the recombinants $T^+L^+S^r$ and Try^+S^r were selected and their sensitivity to phage F2 determined. All the selected recombinants were sensitive to this phage, indicating that the state of the F factor in cells of clones 14-P and 4-P was not defective, but autonomous, and that the frequency of its transmission to recipient cells was high.

It is also clear from the results given in Table 1 that cells of clone cultures 232-P and 18-P had completely lost their donor properties, for the corresponding crosses were sterile. Inability to transfer chromosomes and sensitivity to specific phage demonstrate the presence of a defective sex factor in these cells.

In the next experiments cells of clone cultures 232-P and 18-P, carrying the defective mutant of sex factor, were tested for their ability to act as recipients when crossed with other donors, notably with cells of strains E. coli Hfr C Pro$^-$ and 1485 (F-lac). Fertility of the crosses was judged from the results of selection of $B_1^+S^S$ recombinants, given in Table 2.

As Table 2 shows, the crosses were fertile. Consequently, cells of clone cultures 232-P and 18-P were capable of receiving genetic material from other donors.

To study the multiplication of wild superinfecting sex factor in cells carrying defective sex factor, 16 recombinants were selected from crosses between E. coli 1485 (F-lac) × 232-P, and each of them was recrossed with E. coli P678F$^-$ and J62F$^-$, when the $T^+L^+S^+$ and Try^+S^r recombinants were selected. Fertile combinations resulted in the formation of only two recombinants, with frequencies of appearance of $T^+L^+S^r$ and Try^+S^r recombinants of the order of 0.0012 and 0.00001% respectively.

Hence, in cells carrying a defective mutant of integrated sex factor, multiplication of wild autonomous sex factor is suppressed in most cases.

The presence of cultures of E. coli cells with defective sex factor provides a means of looking for back mutations of sex factor.

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