PROPERTIES OF THE F' FACTORS FORMED IN CROSSES BETWEEN Escherichia coli Hfr DONOR CELLS AND RECOMBINATION DEFECTIVE RECIPIENTS*

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As a result of crossing between Hfr H, KL-96, and KL-99 donor cells with AB 2463 rec A as the recipient, merodiploids carrying factors of different structure (different length) and different activity were isolated: 1) typical F' factors with incorporated proximal chromosomal markers; 2) "long" F' factors of different structure, defective for genes controlling sensitivity to phage f_2; 3) "long" F' factors of different structure defective for genes controlling transfer. Chromosomal markers can be incorporated into the factor regardless of their position relative to sex factor in the original Hfr cells. Defects of the sex factor proper are accompanied by loss of some of its incorporated chromosomal genes, whereas the typical F' factors preserve their structure completely.

KEY WORDS: Escherichia coli; genetic recombination; sex factors; chromosomal genes.

Genetic recombination in Escherichia coli is controlled by a number of genes and complete recombination defectiveness of the bacteria arises only in the case of mutations of the rec A gene [1]. Since it has been shown that crosses between Hfr donor cells and rec A⁻ recipients can be accompanied by the formation of merodiploids carrying sex factor F' [3, 4], this phenomenon was used to determine the size of the donor's chromosomal segments incorporated into the F' factors formed under these conditions, the stability of these combined structures, and the integrity of the sex factor itself contained in them.

In this investigation the test object was F' factors identified in crosses between various strains of Hfr donor cells and recipient cells carrying the rec A_{13} mutation.

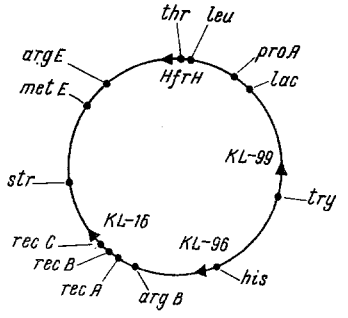

Fig. 1. Chromosome map of E. coli with directions of transfer for donor cells of different Hfr strains. Genetic markers shown outside the circle, names of Hfr strains inside.

EXPERIMENTAL METHOD

In the original crosses the donors were cells of thiamine-dependent, streptomycin-sensitive strains Hfr H, KL-96, KL-99, and KL-16, transferring chromosomes in the directions shown in Fig. 1. The recipient was E. coli strain AB 2463 F⁻ thr leu pro his arg lac str^r rec A_{13}^-.

Crossing was carried out by the standard method by keeping the conjugation mixtures at 37°C for periods excluding the possibility of transfer of the rec A gene. Merodiploids were selected for different markers. Their sensitivity to "male" phage f_2 and "female" phage II was determined by the agar layer method. To determine

* The nomenclature proposed by Demerec et al. [2] is adopted in this paper and abbreviations of the symbols for the genetic markers are taken from Taylor and Trotter [6].

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TABLE 1. Transmissiveness and Chromosomal Transfer Carried out by Identified F' Factors

Merodiploid	Structure of F' factors	Sensitivity to phages		Genetic transfer to J62		Genetic transfer to PA-373					
		f_2	ΦII	episomal marker	chromosomal marker	episomal markers					chromosomal marker
				Pro^+	Try^+	Thr^+	Leu^+	His^+	Arg^+		Met^+
P27	F'-Pro+ Leu+ Thr+	+	−	$52 \cdot 10^{-4}$	$2 \cdot 10^{-1}$	$3 \cdot 10^{-3}$	$2 \cdot 10^{-3}$				$1 \cdot 10^{-1}$
P29	F'-Pro+ Leu+ Thr+	+	−	$67 \cdot 10^{-4}$	$2 \cdot 10^{-1}$	$12 \cdot 10^{-3}$	$14 \cdot 10^{-2}$				$17 \cdot 10^{-1}$
P30	F'-Pro+ Leu+ Thr+	+	−	$76 \cdot 10^{-4}$	$1 \cdot 10^{-1}$	$8 \cdot 10^{-3}$	$1 \cdot 10^{-2}$				$< 1 \cdot 10^{0}$
P10	F'-Pro+ Leu+ Thr+	+	−	$2 \cdot 10^{-3}$	$1 \cdot 10^{-1}$	$8 \cdot 10^{-1}$	$8 \cdot 10^{-1}$				$< 1 \cdot 10^{0}$
P20	F'-Pro+ Leu+ Thr+	+	−	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$				$< 1 \cdot 10^{0}$
P31	F'-Pro+ Leu+ Thr+	+	−	$2 \cdot 10^{-2}$	$< 1 \cdot 10^{0}$	$7 \cdot 10^{-1}$	$< 1 \cdot 10^{-1}$				$< 1 \cdot 10^{0}$
H1	F'-His+ Pro+	+	−	$16 \cdot 10^{-1}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$22 \cdot 10^{-1}$			$< 1 \cdot 10^{0}$
P72	F'-His+ Pro+ Leu+ Thr+	+	−	$6 \cdot 10^{-2}$	$< 1 \cdot 10^{0}$	$3 \cdot 10^{-1}$	$1 \cdot 10^{-1}$	$1 \cdot 10^{-1}$			$< 1 \cdot 10^{0}$
P120	F'-Pro+ Leu+ Thr+ Arg+	+	−	$28 \cdot 10^{-3}$	$1 \cdot 10^{-1}$	$13 \cdot 10^{-1}$	$5 \cdot 10^{-1}$		$2 \cdot 10^{0}$		$< 1 \cdot 10^{0}$
P42	F'-His+ Pro+ Leu+ Thr+	+	−	$2 \cdot 10^{-3}$	$< 1 \cdot 10^{0}$	$5 \cdot 10^{0}$	$2 \cdot 10^{-1}$	$3 \cdot 10^{-1}$			$2 \cdot 10^{0}$
P35	F'-His+ Pro+ Leu+ Thr+	+	−	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$	$< 1 \cdot 10^{0}$			$< 1 \cdot 10^{0}$
H18	F'-His+	+	−		$< 1 \cdot 10^{0}$			$2 \cdot 10^{-2}$			$< 1 \cdot 10^{0}$

segregation of the donor's markers in the merodiploids after cultivation for 18 h in liquid medium without growth factors controlled by the donor genes, they were seeded into nutrient broth (MPB) and cultivated in it for a further 3 h at 37°C. Seedings from the MPB were then obtained on solid nutrient media containing growth factors and the plates were incubated at 37°C for a further 48 h. One hundred colonies of each merodiploid were selected and subcultured on solid media without growth factors and with one of the growth factors. The results were read after 48 h. The transmissiveness of the F' factors formed and their ability to carry out chromosomal transfer were determined in crosses between the merodiploids and J62F⁻ pro try his gal Str^r and Pa-373F⁻ arg met thr leu his lac nal^r Str^r recipients.

EXPERIMENTAL RESULTS

Cross Hfr H × AB 2463. Pro^+ Str^r and His^+ Str^r merodiploids were selected from these crosses.

Of the 117 Pro^+ Str^r merodiploids isolated, 83 were at the same time Thr^+ Leu^+. Having selected 50 such merodiploids they were tested for the presence of sex factor by determining their sensitivity to phages f_2 and ΦII. The tests showed that 38 merodiploids were sensitive to phage f_2 and resistant to phage ΦII, whereas 12 merodiploids were resistant to phages of both types. To obtain information showing that the sex factor and donor Pro^+, Thr^+, and Leu^+ markers in the merodiploids were in an autonomous state, they were tested for their ability to segregate for these markers. The results showed that the frequency of segregation for all markers in all merodiploids was 2-26%. To determine the ability of the merodiploids to carry out genetic transfer, six merodiploids differing in their phage-sensitivity (P27, P29, P30, P10, P20, P31) were investigated selectively. They were crossed with recipient cells I62 and PA-373. The results of the crosses are given in Table 1.

As Table 1 shows, the highest frequency of transmission of the episomal and chromosomal markers was observed in merodiploids sensitive to phage f_2 and resistant to phage ΦII. So far as merodiploids resistant to both phages are concerned, only F' factor was transferred from them to the recipient cells and, moreover, with a low frequency or not at all. Consequently, the F' factors in merodiploids sensitive to phage f_2 and resistant to phage ΦII are typical F'-Pro^+ Leu^+ Thr^+ factors, whereas the F' factors in merodiploids resistant to both phages are F' factors of the same structure, but which have lost during their formation the gene or genes responsible for phage sensitivity and the formation of F-fimbria. As a result, they have lower transmissiveness and do not mobilize the chromosome for transfer.

Of the 76 isolated His^+ Str^r merodiploids isolated, 53 were simultaneously Thr^+ Leu^+ Pro^+. All were resistant to phages f_2 and ΦII, and the frequency of segregation of these markers was 1-4%. Determination of the ability of these merodiploids to carry out genetic transfer showed that they had no such ability. Only the H1 merodiploid could transfer sex factor, but not the chromosome, and this only with low

frequency (Table 1). Consequently, the F' factors in these merodiploids have the F'-His$^+$ Pro$^+$ structure and a defect in the gene (genes) determining phage sensitivity (the formation of F-fimbria). Ability to carry out genetic transfer also was present.

Cross KL-96 × AB 2463. Merodiploids from these crosses were selected for the Pro$^+$ Str^r marker. Altogether 90 Pro$^+$ Str^r colonies were selected, of which 39 were simultaneously Leu$^+$ Thr$^+$ Arg$^+$, 41 were Leu$^+$ Thr$^+$, and 10 were His$^+$ Leu$^+$ Thr$^+$. All these merodiploids were sensitive to phage f_2 only and the frequency of segregation of their donor markers was 2-30%. Ability to carry out genetic transfer was detected in merodiploids P72 and P120, carrying F' factors of different structure (F'-His$^+$ Pro$^+$ Leu$^+$ Thr$^+$ and F'-Pro$^+$ Leu$^+$ Thr$^+$Arg$^+$, respectively). The results in Table 1 show that they had characteristically low donor ability. Clearly these merodiploids carry "long" F' factors, but the sex factor itself in these structures carries a defect in the gene (genes) responsible for transfer.

Cross KL-99 × AB 2463. Selection of presumed merodiploids from these crosses was carried out for Pro$^+$ Str^r and His$^+$ Str^r markers. Of 42 Pro$^+$ colonies three were simultaneously His$^+$ Leu$^+$ Thr$^+$, six were Leu$^+$ Thr$^+$ Arg$^+$, 24 were Leu$^+$ Thr$^+$, and nine were Pro$^+$ only. Nine F'-Pro$^+$ Leu$^+$ Thr$^+$, three F'-Pro$^+$ Leu$^+$ Thr$^+$ Arg$^+$ merodiploids, and one Pro$^+$ merodiploid were sensitive to phage f_2. The remaining 29 merodiploids were resistant to both phages f_2 and ΦII. Segregation of donor markers was carried out only in merodiploids sensitive to phage f_2, in which its frequency was 1-6%. Donor ability was detected in merodiploids P42 and P35 (Table 1). As the results of the tests showed, merodiploids sensitive to phage f_2 carry out episomal and chromosomal transfer, but at reduced frequency. The f_2 ΦII^r merodiploids carried out neither episomal nor chromosomal transfer.

Of the 30 merodiploids selected for the His marker 27 inherited this marker alone and three were simultaneously Arg$^+$. All these merodiploids were sensitive to phage f_2 only, and segregation of donor markers amounted to 1%. Genetic transfer carried out by merodiploids of this type (H18) was extremely low (Table 1). Clearly in these crosses at least factors F'-Pro$^+$ Leu$^+$ Thr$^+$, F'-Pro$^+$ Leu$^+$ Thr$^+$ Arg$^+$, F'-Pro$^+$ His$^+$ Leu$^+$ Thr$^+$, F'-His$^+$, and F'-His$^+$ Arg$^+$, with defects in the genes responsible for transfer, were identified.

Cross KL-16 × AB 2463. From these crosses selection of presumed merodiploids was carried out for Pro$^+$, Leu$^+$, and Thr$^+$ markers, but all the 45 Pro$^+$ colonies, 42 Leu$^+$ colonies, and 39 Thr$^+$ colonies were sensitive to phage ΦII, did not give segregation of the markers, and did not possess donor ability. As was to be expected, all were recombinants of the classical type and their appearance could be explained only by early transfer of the rec A gene.

To study the stability of the F' factors identified in the Hfr H × AB 2463 cross, some of them were introduced into J62F$^-$ cells, after which the latter were used as donors in crosses with AB 1157 recipient cells. The results of these experiments showed that factor F'-Pro$^+$ Leu$^+$ Thr$^+$ preserves its structure, whereas F' factors with analogous structure, but arising from merodiploids resistant to "male" and "female" phages, lost some of the markers.

It can be concluded from the results of these experiments as a whole that different F' factors are formed in rec A$^-$ cells receiving genetic material from donors, namely: 1) typical F' factors with incorporated proximal chromosomal markers; 2) "long" F' factors of different structure defective for genes controlling sensitivity to phage f_2; 3) "long" F' factors with different structure and with a defect of the genes controlling transfer.

Chromosomal markers can be incorporated into a factor irrespective of their position relative to sex factor in the original Hfr cells [5]. Finally, the results show that defects in the genic system of the sex factor proper are accompanied by loss of some of the chromosomal genes incorporated into it.

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