INHIBITION OF MULTIPLICATION OF AN F-LAC FACTOR IN HFR CELLS OF ESCHERICHIA COLI K-12

John Scaife and Julian D. Gross

M.R.C. Microbial Genetics Research Unit
Hammersmith Hospital, London W.12.

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The donor property in Escherichia coli K-12 is determined by the sex factor, F. The sex factor exists in the autonomous state in F^+ donors and is transferred by them at a high frequency to F^- (recipient) cells (Lederberg et al., 1952; Hayes, 1953). In Hfr donors F is integrated on the chromosome and behaves as a terminal marker transferred only rarely. It has been argued from these observations that Hfr cells do not harbour F in the autonomous state (Jacob et al., 1960). However recent studies suggest that Hfr cells, unlike F^+, transfer little or no cytoplasmic material (Fisher, 1962). Fisher has therefore pointed out that, although undetected by transfer, autonomous F may exist in Hfr cells.

By infecting genetically similar Hfr, F^+ and F^- strains with an F-lac factor (Jacob and Adelberg, 1959) and analysing the clones formed, we have been able to demonstrate that multiplication of the F-lac is inhibited in Hfr cells.

Materials and Methods

Bacterial strains. Each of the strains used as recipient had the genotype $T^-L^-B_1^-lac^-$ gal^+ $T6^rS^r$. The F^- strain was a gal^+ revertant of strain $W945F^-$ (Cavalli and Jinks, 1956). The F^+ strain was obtained from this by infection. The Hfr strain was a gal^+S^r recombinant from the cross $W945F^-S^r$ x Hfr Cavalli S^s (Cavalli and Jinks, 1956). The donor strain employed was $W1485(F\text{-}lac)M^-S^s$, obtained by infecting $W1485F^-$ (a non-lysogenic derivative

of 58-161F$^-$) with a lac$^+$ revertant of the F-lac$^-$ carried by strain 2310S (Jacob). It behaves like a typical intermediate male (Adelberg and Burns, 1960), transferring F-lac with a considerably higher frequency than chromosomal markers near the origin, such as T$^+$L$^+$ and P$^+$.

A T$^+$L$^+$P$^-$T6^s derivative of W945F$^-$ was employed in some tests.

Abbreviations for the genetic markers are given under Table 1.

Media and culture methods were as described in de Haan and Gross (1962).

Table 1

Lac$^+$S^r and T$^+$L$^+$S^r recombinants from crosses between W1485(F-lac) donor (S^s) and Hfr, F$^+$ and F$^-$ recipients (S^r).

Recipient	Frequency of T$^+$L$^+$S^r recombinants per donor cell	Frequency of lac$^+$S^r recombinants per donor cell	Appearance of lac$^+$S^r colonies
Hfr	4.8×10^{-4}	3.5×10^{-2}	Variegated
F$^+$	4.2×10^{-4}	1.7×10^{-2}	Mainly variegated
F$^-$	2.9×10^{-2}	1.0×10^{0}	Pure

Young broth cultures (2×10^8 cells/ml) were mixed in equal volumes at 37°C. After two hours, samples were plated on minimal medium supplemented with B_1 (10^{-3} diln.) and on EMB lactose medium (10^{-5} diln.). All plates contained 250µg/ml streptomycin to kill the W1485 donor.
Abbreviations used are: M, methionine; B_1, vitamin B_1; T, threonine; L, leucine; P, proline; S^r,S^s, streptomycin resistance, sensitivity; T6^r,T6^s, T6 resistance, sensitivity; lac,gal, lactose, galactose fermentation.

Results

From Table 1 it may be seen that in each cross the number of lac$^+$ colonies was about fifty times greater than the number of T$^+$L$^+$ recombinants. This difference reflects the much higher frequency with which an intermediate donor strain transfers its autonomous F factor as compared with early chromosomal markers, and demonstrates that the lac$^+$ colonies are derived in each case from cells which received the F-lac factor. The greatly reduced

yield in the crosses involving Hfr and F^+ recipients affects lac^+ and T^+L^+ recombinants equally, and is therefore due simply to the poor efficiency with which such cells act as recipients (Hayes, 1953).

Table 2

Properties of lac^+S^r colonies obtained by infection

Origin of lac^+S^r colonies	1. Acridine orange treatment		2. Donor efficiency		
	Number tested	Number segregating lac^- cells	Number tested	Average frequency of $T^+L^+P^+$ recombs.	Average frequency of $T^+L^+lac^+$ recombs.
Hfr	28	0	15	8.2×10^{-2}	9.0×10^{-2}
F^+	25	24	9	3.4×10^{-2}	8.6×10^{-1}
F^-	25	25	10	2.4×10^{-2}	6.8×10^{-1}

1. Purified colonies were subjected to acridine orange treatment as described by Hirota (1960). After 22 hours' incubation in acridine orange, cells were streaked on EMB lactose medium to detect lac^- segregants.

2. Young broth cultures from some of the purified colonies were mixed with $W945F^- P^-lac^-T6^S$ for 2 hours; $T^+L^+lac^+$ and $T^+L^+P^+$ recombinants were selected on supplemented minimal plates.

Purified lac^+ colonies from each type of cross were examined for possession of F-lac by treatment with acridine orange, which eliminates autonomous F factors (Hirota, 1960). From Table 2 it may be seen that virtually all the lac^+ derived from infection of F^+ and F^- cells are susceptible to acridine orange treatment, whereas those derived from the Hfr are unaffected. This indicates that the latter do not harbour F-lac, but instead must result from recombination between the F-lac and the homologous lac^- segment of the bacterial chromosome. The donor properties of these colonies bear out this conclusion (Table 2). The lac^+ derivatives of the Hfr transfer lac^+ at the same frequency as the chromosomal marker P^+. The derivatives of the F^+ and F^-, on the other hand, behave like intermediate donors, transferring lac^+ at a considerably higher rate than P^+. Moreover, the lac^+ colonies from the crosses employing the Hfr lac^+ derivatives as

donors are all F^- and have generally inherited the closely linked P^+ and $T6^r$ markers. By contrast, those receiving lac^+ from donors of F^+ or F^- origin are themselves intermediate males and have not inherited markers linked to the lac locus.

We may conclude, therefore, that the F-lac factor is unable to multiply in Hfr cells. At some time after entry the F-lac recombines with the bacterial chromosome to give rise to a stable lac^+ subclone, and the lac^+ colony formed is variegated (see Table 1). Like normal F, the F-lac, on entering an F^- cell, multiplies faster than its host and consequently the lac^+ colony produced is pure. The predominantly variegated appearance of the lac^+ colonies resulting from the infection of the F^+ cells indicates that in this case the normal F factors already present in the cell have some inhibitory effect on the multiplication of the F-lac, preventing them from multiplying sufficiently rapidly to enter all progeny cells. This phenomenon demonstrates the existence of a control mechanism regulating the multiplication of autonomous F factors in addition to the one present in Hfr cells which inhibits multiplication completely. In preliminary attempts we have been unable to isolate cells harbouring normal F and F-lac jointly, but it may be mentioned that Stouthamer and de Haan (pers.comm.) have obtained strains harbouring both F-lac and F-gal factors.

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