CO-OPERATION OF TWO SEX FACTORS IN ESCHERICHIA COLI K12

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Received October 7, 1963

The discovery of the fertility factor or sex factor F in Escherichia coli K12 opened up a whole new field of genetic research which has been the subject of a recent monograph (Jacob and Wollman 1961). Other extra-chromosomal elements such as the genetic factor responsible for the maintenance of colicin I, col I have also been shown to have properties characteristic of sex factors. Cells harbouring col I have been shown to pair or conjugate with other cells, leading to the transfer of other extra-chromosomal elements, and in some instances of chromosomal markers, both in Salmonella (Ozeki and Howarth 1961) and in E.coli K12 (Clowes 1961). Yet other genetic elements, the resistance transfer factors or RTFs (see Watanabe 1963) have more recently been shown to have similar properties (Sugino and Hirota 1962).

The interaction and co-operation in E.coli K12 of two of these sex factors, F and col I is reported here.

MATERIALS AND METHODS

Bacterial strains. 58-161 F^-, a methionineless auxotroph of K12 which is streptomycin sensitive (str-s) and does not carry any sex factor. Hfr Cavalli, a derivative of this strain, in which an introduced F sex factor has been integrated on the chromosome, by which the strain is capable of chromosomal transfer at high frequency in an oriented manner as shown in Fig 1. J62F^-, a triple auxotroph of K12 requiring proline,(pro) histidine (his), and tryptophan (try), which

is streptomycin resistant, (str-r) and devoid of F (F^-). Cys 36 col I^+, a strain of Salmonella typhimurium LT2, carrying the sex factor col I (Ozeki, Stocker and Smith 1962).

Male-specific phage. Phage μ which absorbs to and lyses only E.coli K12 strains which are genetic males and harbour F, either in the autonomous (F^+), or integrated (Hfr) state (Dettori, Maccacaro and Piccinin 1961).

Crosses were performed by mixing log. cultures of parental strains at 2×10^8 cells per ml. in equal volumes at 37° and maintaining them at this temperature for two hours. They were then diluted and plated on appropriately supplemented minimal agar plates.

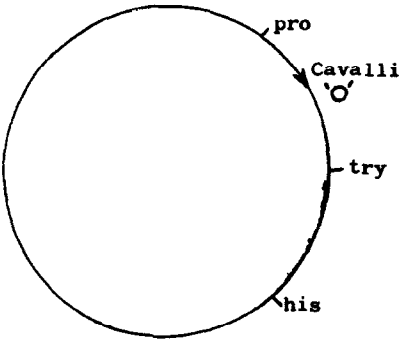

FIGURE 1. Orientation of selected markers in Hfr CAVALLI

The arrow head at O represents the 'origine' and orientation of chromosomal transfer.

RESULTS

Hfr Cavalli was serially sub-cultured for three successive days in broth containing 10^{10} particles per ml. of male-specific phage μ, the survivors being isolated by plating on nutrient agar. All the colonies examined (42) were completely resistant to the phage, producing no plaques when used as indicator to phage suspensions of 10^{10} particles. The parental Hfr Cavalli when mated with J62 produced recombinants at high frequency

and with the characteristic polarity of transfer (Table 1, line 1). On superficial examination the male-specific, phage-resistant mutants (Cavalli MS-r) appeared to be non-fertile. However more precise experiments showed that all possessed a low level of fertility which in most cases gave rise to about 1 recombinant per 10^8 parental cells (line 2). Some isolates tested later, although completely phage resistant showed fertility up to about 5% that of Hfr Cavalli.

TABLE 1: Fertility of Hfr Cavalli and related donors with J62F$^-$
($\sigma^\prime$ met$^-$ str-s x pro$^-$his$^-$try$^-$ str-r F$^-$)

Donor strain	Recombinant Fraction		
	pro$^+$	his$^+$	try$^+$
1. Hfr Cavalli	220×10^{-4}	3.3×10^{-4}	1.2×10^{-4}
2. Cavalli MS-r1	1.3×10^{-8}	0.6×10^{-8}	$<10^{-8}$
3. Cavalli MS-r1 col I$^+$	746×10^{-8}	4.2×10^{-8}	5.1×10^{-8}
4. 58-161 col I$^+$ F$^-$	$c.0.2 \times 10^{-8}$	$c.0.2 \times 10^{-8}$	$c.0.2 \times 10^{-8}$

These MS-r strains appeared to have residual fertility and had not therefore become F$^-$. This was confirmed when it was found that they exhibited an immunity to superinfection by autonomous F prime factors, characteristic of donor strains (Scaife and Gross 1962). Cytological observations revealed that they did not form clumps or pairs when mixed with F$^-$ recipient strains, from which it was inferred that the mutation producing the cell-surface change leading to inability to adsorb the male-specific phage had also led to an inability to form specific male to female contacts. One such "impotent" strain MS-r1, was infected with the col I factor by mixed overnight growth with strain cys 36 col I.$^+$ The mixed cells were differentiated by plating on EMB lactose agar and a Lac$^+$ col I$^+$ colony isolated. This strain, (Cavalli MS-r1 col I$^+$) was identical to the K12 strain in all other properties of auxotrophy, and sensitivity to T phages and λ vir phage and retained resistance to the male-specific phage.

It was similarly crossed with J62F$^-$ with the result shown in line 3, where it can be seen that partial restoration of fertility has occurred. A similar col I$^+$ derivative isolated from the related methionineless strain 58-161F$^-$ was also similarly mated, (line 4).

DISCUSSION

The introduction of the sex factor col I into an F$^-$ strain of E coli results in a strain capable of chromosomal transfer at a low rate, and with the same efficiency for all markers studied (line 4; Clowes 1961). In contrast, when col I is introduced into an "impotent" male strain, genetic transfer by this strain is increased almost a thousandfold above that of either the "impotent" male, or a col I F$^-$ strain and moreover some markers (pro$^+$) are now transferred more than a hundred times more frequently than others (try$^+$). Such a polarity is reminiscent of the original parental Hfr Cavalli, from which is inferred that the chromosomal transfer is mediated by the originally integrated F sex factor. This factor is thought to be present in these MS-resistant cells in an altered form, being now incapable of producing the male-specific F surface, although retaining the characteristic F immunity. The introduction of the col I sex factor, is thought to permit production of the col I mating surface (which in these and in other strains does not permit adsorption of the male-specific phage) restoring fertility to a great extent. The new level of fertility although greatly increased is still however less than a thousandth as efficient as the original Hfr.

This may be due in part to the low frequency of col I mediated contacts which by cytological observation are seen to be only 2 to 5% that of Hfr, in which almost all cells rapidly form contacts. However, even after correcting for this, the efficiency of chromosomal transfer per conjugating cell is still only 1 to 2% that of the original Hfr. Jacob, Brenner and Cuzin (1963) have recently suggested that the F factor is located on the cell surface and is directly involved in the localised

production of male-specific antigen. Mating contacts are suggested to occur only at these specific sites which then trigger off chromosomal transfer. Since the introduction of col I into F^+ cell is found not to destroy either the ability to adsorb male-specific phage, or to form contacts with high efficiency, it may be assumed that the col I specific surface is not superimposed on that of the F. Col I specific contacts are therefore likely to be made at sites other than F sites. However, it is possible that these col I contacts may trigger off (with a low probability of 1 to 2%) specific F mediated transfer at neighbouring sites. Other explanations however are not at all excluded.

The transient association of sex factors with the chromosome, postulated to account for chromosomal transfer by Sugino and Hirota (1962), may however not always be essential. In our experiments the function of col I is interpreted as merely producing cell conjugation, genetic transfer being mediated by other means. When similarly introduced into an F^- cell, genetic transfer could result from rare spontaneous chromosomal breaks creating a linear structure, and in cells carrying an affinity locus for an alternative sex factor such as the Richter "female-3" strain, such breaks are likely to be more probable in the vicinity of this locus which would lead to polarised transfer. In either case the function of the infecting sex factor might be merely to provide physical conjugation without supposing chromosomal association or attachment.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the efficient technical assistance of Mr. E.E.M.Moody.

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