Deletion of chromosomal markers in association with F-prime factor formation in *Escherichia coli* K12

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Genetic donors of *E. coli* K12 harbour the sex factor (F) which may be in two states with respect to the circular chromosome of the bacterial cell. F^+ cells harbour F in the autonomous state, whilst Hfr strains carry it integrated in the chromosome. As a result of having the sex factor in the integrated state, Hfr donors are able to transfer the chromosome to recipient (F^-) cells as an oriented linear structure, the orientation being determined by the site at which integration has occurred (Wollman, Jacob & Hayes, 1956; Jacob & Wollman, 1958).

An integrated sex factor may return to the F^+ state; in addition it may be released from the chromosome as a variant, F-prime factor, in which the sex factor is associated with the region of the chromosome which lay next to it in the ancestral Hfr strain. On transfer to an F^- strain, an F-prime factor remains largely in the autonomous state. A proportion of cells, however, have the F-prime factor integrated in the chromosome as a result of a genetic exchange between its chromosomal fragment and the specific region of the bacterial chromosome with which it has homology (Scaife & Gross, 1963; Cuzin & Jacob, 1963). Consequently a culture of an F-prime donor is observed to transfer its chromosomal markers with the orientation of the parental Hfr strain (Adelberg & Burns, 1960).

The strain W3747 (F13) arose as a subclone of an Hfr donor (Hfr 13) which transfers gal^+ as a proximal marker during conjugation, lac^+ entering on its distal segment.† It was shown to transfer the genes ad^+, $T6^r$ and lac^+ together with the sex factor at a frequency much higher than that of other markers and was therefore concluded to harbour an F-prime factor (F13) carrying these genes (Hirota & Sneath, 1961).

We have observed that chromosome transfer by the strain W3747 (F13) differs markedly from that of the typical F-prime donor described above. The difference is concluded to be a consequence of the mechanism of F-prime factor formation.

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† The genetic markers mentioned in this report are arranged in the following order on the *E. coli* chromosome:

$$—thr—leu—pro—lac—T6—ad—gal—try—his—$$

The abbreviations used are: *ad*, adenine; B_1, vitamin B_1; gal^+/gal^-, ability/inability to ferment galactose; *his*, histidine; lac^+/lac^-, ability/inability to ferment lactose; *leu*, leucine; *met*, methionine; *pro*, proline; S^r/S^s, resistance/sensitivity to streptomycin; *thr*, threonine; *try*, tryptophan; $T6^r/T6^s$, resistance/sensitivity to bacteriophage T6; λ^+/λ^-, lysogenic/non-lysogenic for the temperate bacteriophage λ; O, the leading extremity of the structure transferred by an Hfr cell to a recipient during conjugation.

METHODS AND MATERIALS

Media and culture methods have been described elsewhere (de Haan & Gross, 1962; Scaife & Gross, 1963).

Bacterial strains. Donors: Hfr 13 $\lambda^- met^-$ (a λ^- derivative of the strain W3200 (Hirota & Sneath, 1961), which during conjugation transfers its chromosomal markers in the order $O\text{-}ad\text{-}gal\text{-}try \ldots pro\text{-}lac\text{-}F$ (see Broda, Beckwith & Scaife, 1964); W3747 $met^-T6^r\lambda^+$ (Hirota & Sneath, 1961); W1655 met^-S^s (F-lac^+) (Scaife & Gross, 1963).

Recipients: W945 $thr^- leu^- B_1^- gal^- S^r F^-$ (Cavalli & Jinks, 1956); W1655 $met^-\lambda^- F^-$ (isolated by Dr R. C. Clowes as an F^- derivative of W1655 $met^-\lambda^- F^+$ (Lederberg & Lederberg, 1953)); J62 $pro^- try^- his^- lac^- S^r F^-$ (Clowes & Rowley, 1954); 1177 $ad^- B_1^- lac^-$ (isolated by Dr P. G. de Haan).

RESULTS

Unlike a typical F-prime donor the strain W3747 (F13) transfers chromosomal markers with a very low efficiency. It may be seen from Table 1 that whilst it donates lac^+ to a high proportion (61%) of recipient cells on the autonomous F13 factor, only about 0·004% of the F^- cells inherit the donor marker gal^+ after mating for 1 hour. The fact that the $thr^+ leu^+$ recombinant class is recovered with about the same efficiency shows that a culture of the strain does not contain a class of donors transferring their chromosomal markers with the same orientation as the ancestral Hfr. It is concluded from these observations that the F13 factor does not readily integrate at its ancestral site on the W3747 chromosome.

Table 1. *Chromosomal mobilization by the F13 factor*

One ml. aliquots of young broth cultures of the two donor strains were each mixed with 1 ml. of a young broth culture of W945 $thr^- leu^- B_1^- lac^- gal^- S^r F^-$ and gently aerated for 1 hour at 37° before diluting and spreading on minimal medium appropriately supplemented for the recovery of the three recombinant classes.

	Percentage of recipient cells yielding		
Donor strain	$thr^+ leu^+ S^r$ recombinants	$gal^+ S^r$ recombinants	$lac^+ S^r$ recombinants
W1655 $met^- S^t$ (F13)	0·003	2·0	17·0
W3747 $met^+ S^t$ (F13)	0·001	0·004	61·0

The F-13 factor itself can give chromosomal donors transferring their markers with the orientation of the ancestral Hfr. In Table 1 the donor properties of a strain (W1655) infected with the F13 factor are compared with those of W3747 (F13). It can be seen that when harboured by the strain W1655 the F13 factor permits gal^+ transfer at a frequency almost 1000-fold greater than that of the thr^+ and leu^+ markers, showing that the F-prime factor is able to promote chromosome transfer with the orientation of the Hfr strain from which it was derived. The result indicates that the inability of the strain W3747 (F13) to transfer its chromosomal markers with the normal efficiency and orientation is due to a specific deficiency of this strain.

There is little doubt that the physiological requirements for integration are met in the strain W3747 (F13), since some transfer of thr^+, leu^+ and gal^+ is observed. It is therefore reasonable to suppose that the absence of a uniform class of chromosomal donors carrying the F13 factor at the ancestral Hfr site is due to a lack of homology between the W3747 chromosome at this site and the chromosomal fragment of the F13 factor, suggesting that

the region corresponding to the fragment is deleted from the chromosome. Recent results indicate that deletions of this type do prevent integration of an F-prime factor at its normal site (Cuzin & Jacob, 1964; Pittard & Ramakrishnan, 1964).

On the above hypothesis the strain W3747 (F13) would remain entirely haploid, some of its functions being determined solely by the genes carried on the F13 factor. Should one of these genes be essential for the viability of the cell, loss of the F13 factor would be lethal. Consequently, it should be impossible to select F$^-$ subclones from W3747

Table 2. *The response of W3747 (F13) to subculture in acridine orange*

Stationary cultures of W3747 (F13) and W1655 (F-*lac*$^+$) were diluted in broth to give 10^5 cells per ml. One tenth of a millilitre of these suspensions was then inoculated into 1 ml. of broth containing 50 μg/ml. of acridine orange. The broth used in this experiment was adjusted to pH 7·6. The cultures were grown overnight and single colonies were isolated. The colonies were then tested for the ability to transfer *thr*$^+$, *leu*$^+$ and *lac*$^+$ to a recipient strain as before (Table 1).

	W3747 (F13) Subcultured in broth		W1655 (F-*lac*$^+$) (control) Subcultured in broth	
	+ Acridine orange	− Acridine orange	+ Acridine orange	− Acridine orange
Number of colonies which are F$^-$	0	0	14	0
Number of colonies which transfer *lac*$^+$ at a high rate	1	10	0	10
Number tested	25	10	14	10

(F13) after treatment with acridine orange, which eliminates autonomous sex factors (Hirota, 1960). The data cited in Table 2 confirm this expectation. It may be seen that under conditions where all the clones reisolated after treatment of the control F-prime strain (W1655 (F-*lac*$^+$)) are F$^-$, none of the W3747 subclones have lost the donor property.

Further, it is expected that only those cells carrying the F13 factor integrated in the chromosome, and therefore resistant to elimination by acridine orange, should show normal growth. As a result the great majority of colonies reisolated after subculture in broth containing the dye should carry the F13 factor in the integrated state.

The donor properties of such subclones differ markedly from those of the untreated strain. They transfer the marker *ad*$^+$ with an efficiency 10-fold less than W3747, whilst *lac*$^+$ transfer by the new donors is reduced by a factor of 10^{-4}. Crosses using one of these isolates (A-1) have shown that the majority of the *ad*$^+$ recombinants formed are F$^-$ and do not lose their *ad*$^+$ phenotype on subculture in acridine orange, indicating that the *ad*$^+$ marker is transferred to recipient cells as a chromosomal gene.

These results may be explained by assuming that the acridine-treated subclones carry the F13 factor integrated into the chromosome in such a way as to give a structure transferring *ad*$^+$ as a proximal marker and carrying the *lac* region of the F13 factor on the extremity transferred last during conjugation. Kinetic studies determining the times of entry of various markers confirm this explanation, the markers of the A-1 donor being transferred in the order *o-ad-pro-try-his-lac-*.

It will be noted that unlike Hfr 13 the W3747 derivative, A-1, transfers *pro*$^+$ as an early marker during conjugation, showing, as expected on our hypothesis, that the F13 factor is integrated at a site unrelated to that at which it arose in the ancestral Hfr strain. Moreover, it may be seen that the *lac*$^+$ genes are missing from their normal position between the *pro* and *try* markers on the chromosome of this strain, providing confirmation that this region is deleted in W3747.

DISCUSSION

A model for the formation of F-prime factors is presented in the foregoing Note (Broda, Beckwith & Scaife, 1964). It proposes that variant sex factors of the F13 type are formed by a reciprocal genetic exchange between two sites of the bacterial chromosome, lying on either side of the sex factor. The two products of such an exchange are visualized as circular structures, one being the F-prime factor itself, which opens to be transferred linearly during conjugation. The other product is a circular bacterial chromosome from which that fragment acquired by the F-prime factor has been deleted.

The strain W3747 (F13) arose as a spontaneous variant of the strain Hfr 13, harbouring the F13 factor (Hirota & Sneath, 1961). Its chromosome may therefore be regarded as the second product of the event which gave rise to the F13 factor, so that the presence of the deletion observed in the *ad-lac* region is consistent with the model suggested for F-prime formation.

In transfer on the autonomous F-13 factor the marker ad^+ enters recipient cells first shortly followed by the lac^+ marker (Hirota & Sneath, 1961). By contrast our kinetic studies on the derived strain A-1 show that after integration of the F13 factor in the chromosome these two markers, although transferred in the same order to recipient cells, are now widely separated; ad^+ lies on the proximal segment of the transferred Hfr chromosome whilst lac^+ is transferred as a distal marker.

This result indicates that the bacterial chromosome has been inserted into the chromosomal fragment of the F13 factor at a site lying between the ad^+ and lac^+ markers. It may be simply explained by assuming that this region of the F-13 factor has limited genetic homology with the site on the bacterial chromosome, permitting occasional synapsis between them, followed by a reciprocal genetic exchange. Such an exchange would lead to the formation of a single structure which during conjugation transfers the ad^+ marker on its proximal extremity and the lac^+ marker on its distal end.

REFERENCES

ADELBERG, E. A. & BURNS, S. N. (1960). Genetic variation in the sex factor of *E. coli*. *J. Bact.* **79**, 321–330.

BRODA, P., BECKWITH, J. R. & SCAIFE, J. (1964). The characterization of a new type of F-prime factor in *E. coli* K12. *Genet. Res.* **5**, 489–494.

CAVALLI-SFORZA, L. L. & JINKS, J. L. (1956). Studies on the genetic system of *E. coli* K12. *J. Genet.* **54**, 87–112.

CLOWES, R. C. & ROWLEY, D. (1954). Some observations on linkage effects in genetic recombination on *Escherichia coli* K12. *J. gen. Microbiol.* **11**, 250–260.

CUZIN, F. & JACOB, F. (1963). Integration reversible de l'episome sexuel F′ chez *Escherichia coli* K12. *C. R. Acad. Sci., Paris*, **257**, 795–797.

CUZIN, F. & JACOB, F. (1964). Deletions chromosomiques et integration d'un episome sexuel *F-lac*⁺ chez *Escherichia coli* K12. *C. R. Acad. Sci., Paris*, **258**, 1350–1352.

DE HAAN, P. G. & GROSS, J. D. (1962). Transfer delay and chromosome withdrawal during conjugation in *E. coli*. *Genet. Res.* **3**, 251–272.

HIROTA, Y. (1960). The effect of acridine dyes on mating type factors in *E. coli*. *Proc. nat. Acad. Sci., Wash.*, **46**, 57–64.

HIROTA, Y. & SNEATH, P. H. A. (1961). F′ and F-mediated transduction in *Escherichia coli* K12. *Jap. J. Genet.* **36**, 307–318.

JACOB, F. & WOLLMAN, E. L. (1958). Genetic and physical determinations of chromosomal segments of *E. coli*. XIIth S.E.B. Symposium. The Biological replication of macromolecules. 75–92.

LEDERBERG, E. & LEDERBERG, J. (1953). Genetic studies of lysogenicity in *E. coli*. *Genetics*, **38**, 51–64.

PITTARD, J. & RAMAKRISHNAN, S. (1964). Gene transfer by F′ strains of *E. coli* K12. *J. Bacteriol.* (in press),

SCAIFE, J. & GROSS, J. D. (1963). The mechanism of chromosome mobilization by an F-prime factor in *Escherichia coli* K12. *Genet. Res.* **4**, 328–331.

WOLLMAN, E. L., JACOB, F. & HAYES, W. (1956). Conjugation and genetic recombination in *Escherichia coli*. *Cold. Spr. Harb. Symp. quant. Biol.* **21**, 141–162.