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SHORT NOTES

The characterization of a new type of F-prime factor in *Escherichia coli* K12

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INTRODUCTION

The sex factor, F, which is harboured by donor cells of *E. coli* K12, can exist either in the autonomous state (in F^+ cells) or integrated in the chromosome (in Hfr cells). During conjugation Hfr strains transfer the chromosome to recipient (F^-) cells in an oriented fashion, the sex factor itself entering on the tail of the structure transferred. Hfr strains can revert to the F^+ state by release of their integrated sex factor from the chromosome. An integrated sex factor may also return to the autonomous state carrying with it chromosomal markers; variant sex factors of this type are termed F-prime factors.

Studies on several F-prime factors have indicated that they carry the segment of the chromosome transferred distally in conjugation by the ancestral Hfr strain (Jacob & Adelberg, 1959; Hirota & Sneath, 1961; Pittard, Loutit & Adelberg, 1963). In this communication we describe the characterization of a new type of F-prime factor carrying segments from both distal and proximal regions of the ancestral Hfr chromosome. A model for F-prime factor formation is presented which accounts for the properties of structures both of this type and those previously described.

METHODS AND MATERIALS

Bacterial strains. The following strains were employed: C600 $thr^- leu^- B_1^- lac^- S^r$ F^- (Appleyard, 1954); an F^- derivative (isolated by Dr R. C. Clowes) of W1655 $met^- S^s F^+$ (Lederberg & Lederberg, 1953); J62 $pro^- try^- his^- lac^- S^s F^-$ (Clowes & Rowley, 1954); X33 $B_1 ad^- ura^- try^- his^- lac^- S^r F^-$ (supplied by Dr S. Brenner); 1177 $B_1 ad^- lac^- S^r$ (supplied by Dr P. G. de Haan); Hfr B11 (Hayes, 1964), a derivative of W1655 F^+, transferring its genes in the order *O-T6-ad-try ... pro-lac*-F; Hfr 13 (Hirota & Sneath, 1961), derived from 58–161 $met^- S^s F^+$. This strain donates its genes during conjugation in the order *O-T6-ad-try ... pro-lac*-F. HfrH $\lambda^- met^- S^s$, which transfers its genes in the order *O-thr-leu-pro-lac-ad-try* ... F (Hayes, 1953).

Abbreviations: ad, adenine; B_1, vitamin B_1; *his*, histidine; lac^+/lac^-, ability/inability to ferment lactose; *leu*, leucine; *met*, methionine; *O*, leading extremity of an Hfr chromosome in transfer; *pro*, proline; S^r/S^s, resistance/sensitivity to streptomycin; $T6^r/T6^s$, resistance/sensitivity to bacteriophage T6; *thr*, threonine; *try*, tryptophan; *ura*, uracil; λ^+/λ^-, lysogenic/non-lysogenic for the temperate bacteriophage λ.

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

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RESULTS

The Hfr strain B11 (Hayes, 1964) transfers the genes for sensitivity to the phage T6 ($T6^s$) and for adenine independence (ad^+) as proximal markers during conjugation, whilst the *lac* region is transferred on the distal segment of the chromosome, and thus only enters a recipient cell after mating has been in progress for about 100 min. (Fig. 2 (A)). This property may be used to isolate F-prime factors arising spontaneously in a culture of the Hfr strain. By isolating lac^+ recombinants after mating the donor for a restricted period (60′) with a lac^- recipient, one may select recombinants which have received the lac^+ marker not by chromosomal transfer but by transfer on an F-prime factor in the autonomous state (Jacob & Adelberg, 1959).

F-prime factors of this type were isolated by mating a young broth culture of the streptomycin-sensitive Hfr strain with a similar culture of the recipient strain C600 $thr^- leu^- B^- lac^- T6^s S^r$. After an hour the mixture was violently shaken to separate mating pairs and diluted 1000-fold into broth containing streptomycin (250 μg./ml.) to kill the donor strain. The treated mixture was grown overnight at 37° C. to allow epidemic spread of the autonomous F-prime particles through the recipient population. After incubation, dilutions of the culture were spread on EMB lactose medium containing streptomycin. On this medium $lac^+ S^r$ recombinants appeared as papillae on a background of recipient ($lac^- S^r$) cells after incubation for 2 days. The recombinants were picked, purified and tested for their ability to transfer the lac^+ property to a lac^- recipient strain. In test crosses against the strain 1177 $B_1^- ad^- lac^- T6^r$ F⁻ it was shown that four of the five recombinants tested transfer lac^+ with a high efficiency and were therefore concluded to harbour F-prime factors carrying the *lac* region.

Examination of the lac^+ recombinants isolated from the test crosses shows that a considerable proportion of them inherit the ad^+ and $T6^s$ markers of the donor strain. This observation suggests that the F-prime factor isolated carries not only the *lac* region of the bacterial chromosome but also the segment bearing the ad^+ and $T6^s$ genes. This conclusion is confirmed by the results of an experiment (Table 1) in which recombinants

Table 1. *The response of F-prime BB1 to acridine orange*

Two of the $lac^+ S^r$ isolates recovered after mating the recipient strain C600 $thr^- leu^- B_1^- lac^- S$ with Hfr B11 (see text) were crossed with the strain 1177 $B_1^- ad^- lac^- S^r$. One $ad^+ lac^+$ recombinant from each cross was picked, purified and inoculated into broth and into broth containing 50 μg./ml. acridine orange at pH 7·6 to give a final inoculum of about 100 cells/ml. After incubation single colonies were reisolated on EMB-lactose medium and the lac^+ and lac^- colonies obtained were tested by replication on to appropriately supplemented minimal medium for retention of the ad^+ phenotype.

		Phenotype of colonies			
		% $ad^+ lac^+$	% $ad^- lac^+$	% $ad^- lac^-$	Total tested
Recombinant I	Subcultured in broth	100	0	0	74
	Subcultured in broth plus acridine orange	77	7	16	92
Recombinant II	Subcultured in broth	100	0	0	37
	Subcultured in broth plus acridine orange	48	7	45	162

were tested for the effect on their lac^+ and ad^+ phenotypes of subculture in acridine orange. Such treatment leads to the loss of autonomous sex factors (Hirota, 1960). It will be observed that a large proportion (70–80%) of those cells losing their lac^+ character

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after subculture in acridine orange also lose the ad^+ phenotype, providing confirmation of our conclusion that both markers are located on the F-prime factor.

Some information on the structure of this new F-prime factor (F-prime BB1) has been obtained by determining the order in which a donor strain carrying the factor transfers the ad^+ and lac^+ markers to a recipient strain.

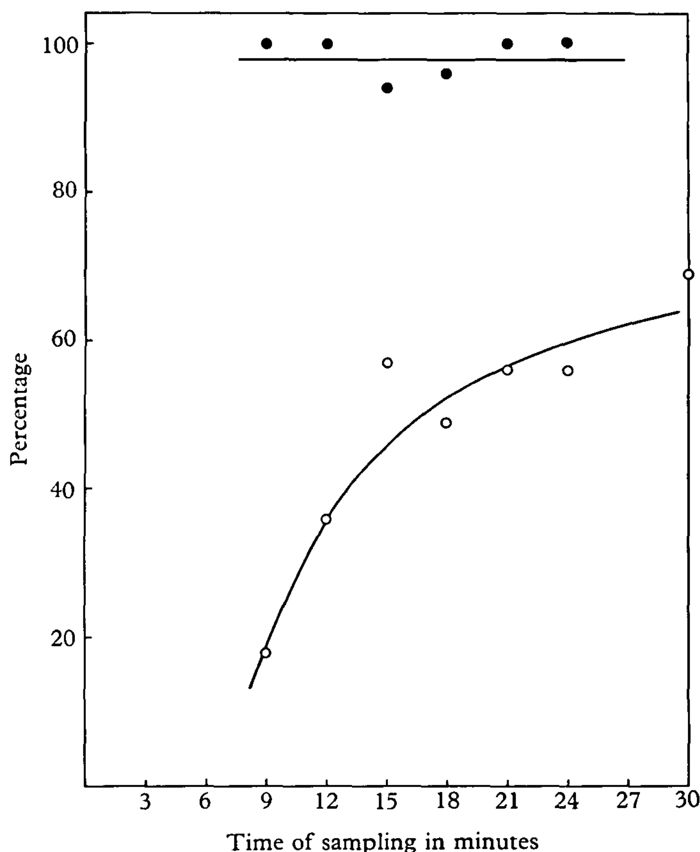

FIG. 1. The order of *ad* and *lac* on F-prime BB1. A young broth culture of W1655 $met^- ad^+ lac^+ S^s$ (F′BB1 $ad^+ lac^+$) was mixed with an equal volume of 1177 $B_1^- ad^- lac^- S^r$. At intervals samples were withdrawn, agitated to separate mating pairs and plated after dilution on to minimal-agar plates, appropriately supplemented for the selection of $ad^+ S^r$ and $lac^+ S^r$ recombinants. The resulting recombinant colonies were then picked, purified and tested for the *lac* and *ad* characters.

○ = percentage of ad^+ recombinants which are lac^+.
● = percentage of lac^+ recombinants which are ad^+.

A donor carrying the F-prime factor was crossed with a $lac^- ad^- S^r$ recipient and the ad^+ and lac^+ recombinants obtained after increasing periods of mating were tested for inheritance of lac^+ and ad^+ respectively (Fig. 1). Fig. 1 shows that the proportion of ad^+ recombinants which are lac^+ increases with increasing time of mating while the proportion of lac^+ which are ad^+ remains constant, showing that ad^+ precedes lac^+ during transfer on the F-prime BB1 factor. Since all the available evidence (Hirota & Sneath, 1961;

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Pittard, Loutit & Adelberg, 1963) indicates that the sex factor is transferred distally on an F-prime factor it is concluded that F-prime BB1 has the structure: *ad-lac*-F.

Studies on the transfer of chromosomal markers by a donor strain harbouring the new F-prime factor have been made. The frequency of transfer of the chromosomal markers pro^+ and try^+ by the strain W1655 (F′ BB1) was compared with their frequency of transfer by the ancestral Hfr strain, B11. In parallel crosses, mating young broth cultures of the two donors with the recipient strain J62 for one hour, it was found that in both cases try^+ was transferred over 100 times more frequently than pro^+, indicating that the F-prime factor causes the chromosome to be transferred with the same orientation as in the ancestral Hfr strain.

There is a clear similarity between the F-prime factor BB1 and the F-13 factor of Hirota & Sneath (1961). Both structures carry the chromosomal markers *T6*, *ad* and *lac*. Kinetic studies have shown that *ad* precedes *lac* in transfer on both structures (see Fig. 1) and that the entry times for these two markers correspond in the two F-prime factors. In addition, the results presented in Table 2 indicate that the sex factors of the two

Table 2. *The transfer of* ad^+ *by Hfr 13*

Young broth cultures of the Hfr strains B11, 13 and H were mated in parallel crosses against strain X33 $B_1^- ad^- ura^- try^- his^- lac^- S^r F^-$ for 1 hour. Dilutions were plated on minimal medium appropriately supplemented for the selection of try^+ recombinants. Colonies were purified on the same selective medium and tested for the *ad* character.

Donor strain	% try^+ which were ad^+	No. of try^+ recombinants tested
Hfr B11	36	194
Hfr 13	57	102
Hfr H (control)	32	79

parental Hfr strains are integrated at closely similar sites. Try^+ recombinants recovered from parallel crosses using Hfr B11 and Hfr 13 as donors were scored for inheritance of the ad^+ marker from the donor. It was expected that if Hfr 13 transfers ad^+ as a proximal marker the proportion of try^+ recombinants inheriting ad^+ would be essentially the same as in the Hfr B11 cross, whereas if Hfr 13 transfers ad^+ as a distal marker virtually none of the try^+ recombinants should be ad^+. The results presented in Table 2 show that Hfr 13 does transfer ad^+ as a proximal marker, leading to the conclusion that the two Hfr strains must both carry their sex factors integrated between *ad* and *lac*.

DISCUSSION

Figure 2 (A–C) shows a formal representation of the events postulated for the formation of an F-prime factor. Firstly, breaks occur at the sites labelled x and y in the figure. Secondly, rejoining of the ends occurs in such a way as to (a) restore the circularity of the bacterial chromosome and (b) give rise to a closed structure, the F-prime factor, which has transfer properties directly analogous with the ancestral Hfr chromosome (Scaife & Gross, 1964).

In Fig. 2 (D) we present a model which would incorporate the above two stages in a single event. If the sites x and y represent regions of limited genetic homology, synapsis between them, followed by a reciprocal genetic exchange at the site of pairing would give rise to the two structures shown in Fig. 2 (C).

The model proposed has the following predictions. Firstly the chromosomal markers of the Hfr strain incorporated into the F-prime factor should retain their original

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orientation of transfer during conjugation. Our observation that the marker ad^+ precedes lac^+ on F-prime BB1 confirms this prediction. In addition, preliminary results indicate that the $T6^s$ marker on F-prime BB1 enters recipient cells before ad^+, as expected on the above model. Secondly, it is predicted that the chromosome in the cell where the

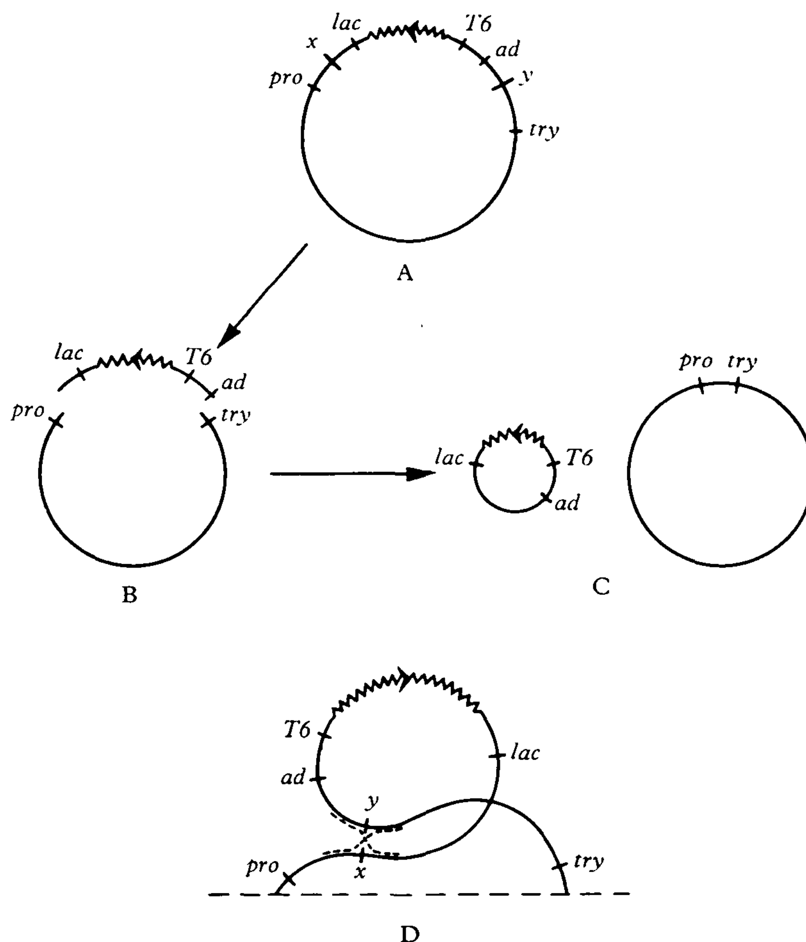

Fig. 2. The mechanism of F-prime formation in *E. coli*. Diagrams A, B, and C represent formally the sequence of events necessary for the formation of F′ BB1 (see text). A → B shows the release of the F factor associated with a piece of the bacterial chromosome. In B → C there is union of the open ends of the resulting structures.

Diagram D shows the model proposed. By assuming a genetic exchange between sites x and y (see text) the above sequence can be expressed as a single event.

The sex factor, represented by a wavy line, is shown inserted in the chromosome (Campbell, 1962). The direction of transfer during conjugation is indicated by the arrow.

F-prime factor arose should have a deletion corresponding to the fragment contained in the new structure. Evidence presented in a separate report (Scaife & Pekhov, 1964) indicates that this is the case. Thirdly, we expect that any Hfr strain with its sex factor

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integrated at a site between the homologous regions x and y could give rise to F-prime factors carrying both *ad* and *lac*. Our results indicate a close similarity between Hfr 13 and Hfr B11. The observation (Hirota & Sneath, 1961) that F13 carries the ad^+ and lac^+ markers is therefore consistent with the above model.

It may be pointed out that the model presented will account for the formation of F-prime factors not carrying the proximal region of the parental Hfr chromosome if it is assumed that the site corresponding to y in Fig. 2 may lie within the sex factor itself. An analogous genetic exchange involving such a site would result in the retention of a fragment of the sex factor by the bacterial chromosome. Such a fragment would correspond to the sex factor attachment locus observed by Adelberg & Burns (1960) and by Richter (1961).

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