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F-prime factor formation in *E. coli* K12

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1. INTRODUCTION

F-prime factors of *E. coli* K12 are composed of the sex factor, F, together with a segment of the bacterial chromosome. They arise in Hfr strains, by release of the integrated sex factor together with the adjacent segment of the chromosome. It has been proposed (Broda, Beckwith & Scaife, 1964) that this process requires a genetic exchange between regions of *limited* homology located in the bacterial chromosome and lying on either side of the integrated sex factor. Such an exchange

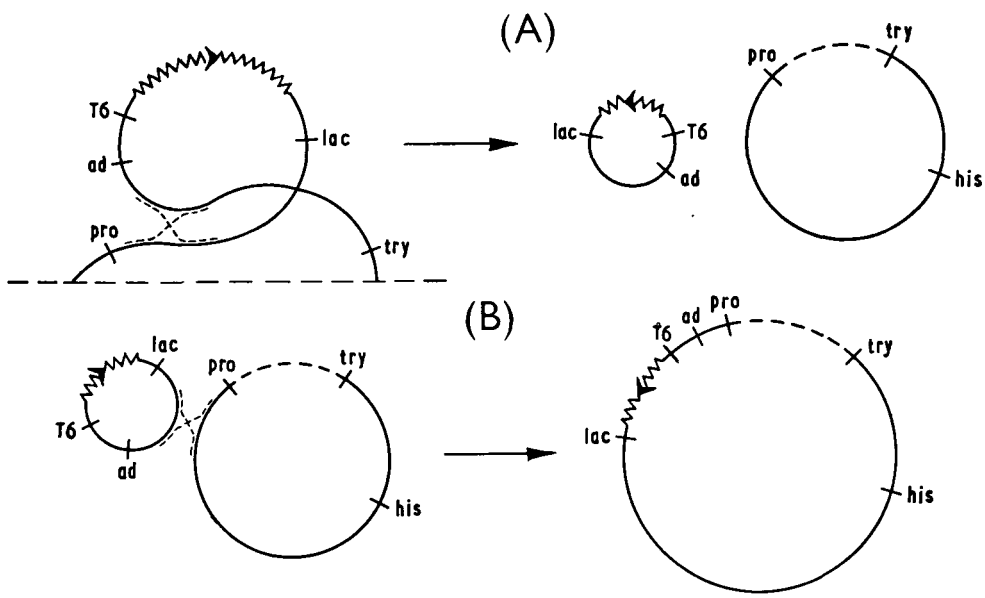

Fig. 1. Genetic interactions in the strain W3747. A. The proposed model for the formation of the F-prime factor F13 (Broda, Beckwith & Scaife, 1964). Regions of homology within the bacterial chromosome are shown aligned to permit the reciprocal exchange between them which would give rise to the F-prime factor. The deletion in the chromosome resulting from this event is shown as a broken line between the markers *pro* and *try*. B. Integration of F13 into the chromosome of W3747. The participating structures are shown aligned so as to give rise to an Hfr strain with the properties of Hfr A1.

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would yield a circular F-prime factor and a chromosome with a deletion corresponding to the fragment incorporated by the F-prime factor (Fig. 1A).

Two aspects of the model are considered in this paper. Firstly, data are presented demonstrating the existence of a deletion in a strain, W3747, where an F-prime factor, F13, has arisen (Hirota & Sneath, 1961; Scaife & Pekhov, 1964). Secondly, evidence has been obtained confirming that, as required by the model, exchanges may occur between chromosomal regions of only limited homology.

In the course of this investigation results were obtained indicating that inhibition of F-prime factor replication does not prevent expression of all genes on the factor.

2. MATERIALS AND METHODS

(i) *Media*

Cells were grown in nutrient broth (Oxoid NB2). Nutrient agar consisted of the same broth solidified with 2% New Zealand agar. The minimal medium used for the selection of recombinants was described by de Haan & Gross (1962). EMB lactose medium was described by Lederberg (1950).

(ii) *Bacterial strains*

(*a*) *Donor strains*

W3747 *met*$^-$**T6*.+*S*. (Hirota & Sneath, 1961); derivatives of W3747 described in the text.

(*b*) *Recipient strains*

J62 *pro*$^-$*try*$^-$*his*$^-$*S*^{r} (Clowes & Rowley, 1954); X33 *ade*$^-$*pro*$^-$*try*$^-$*his*$^-$*ura*$^-$*S*^{r} kindly supplied by Dr S. Brenner); W954 *thr*$^-$*leu*$^-$*B*$_1^-$*lac*$^-$*S*^{r} (Cavalli-Sforza & Jinks, 1956); X139 *pyrD his*$^-$*tyr*$^-$*S*^{r} (kindly supplied by Dr J. R. Beckwith).

3. RESULTS

(i) *Hfr donors isolated from the strain W3747*

The F13 factor incorporates the chromosomal region carrying the genes for lactose fermentation and two of the genes for adenine synthesis (Jacob, Ullmann & Monod, 1965). Thus on our hypothesis, its formation should have resulted in the deletion of these and all other genes in this segment from the chromosome of the strain, W3747, in which it arose. Removal of the F13 factor from this strain should, therefore, yield derivatives unable to perform the functions determined by the genetic region in question. If one or more of these functions were essential for the survival of the cell, removal of the F13 factor would be lethal.

A strain with an autonomous sex factor, growing in broth containing acridine orange (AO broth) gives rise to F$^-$ cells. This is due to inhibition of sex factor multiplication by the dye (Hirota, 1960). If F$^-$ derivatives of the strain W3747

* Abbreviations: *ade*, adenine; *arg*, arginine; *gal*, galactose; *his*, histidine; *lac*, lactose; *leu*, leucine; *met*, methionine; *pro*, proline; *pyr*, pyrimidine; *S*$^{r/s}$, streptomycin resistant/sensitive; *T6*$^{r/s}$, resistant/sensitive to coliphage T6; *thr*, threonine; *try*, tryptophan; *tyr*, tyrosine; *ura*, uracil.

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were not viable, only those cells which had retained the F13 factor after subculture in AO broth would be able to give rise to colonies on transfer to solid medium. After prolonged subculture surviving cells would have a sex factor which had escaped inhibition by the dye. Since sex factors integrated in the chromosome are not subject to acridine inhibition, some of these cells should carry the sex factor in the integrated state.

Data confirming that colonies isolated after subculture in AO broth have retained the donor property have been presented in a previous publication (Scaife & Pekhov, 1964). A study, presented here, of the conjugation properties of three such strains further confirms that they carry the F13 factor in the integrated state. Figure 2 shows the results of interrupted mating experiments demonstrating the order in which they transfer chromosomal markers during conjugation. It will be observed

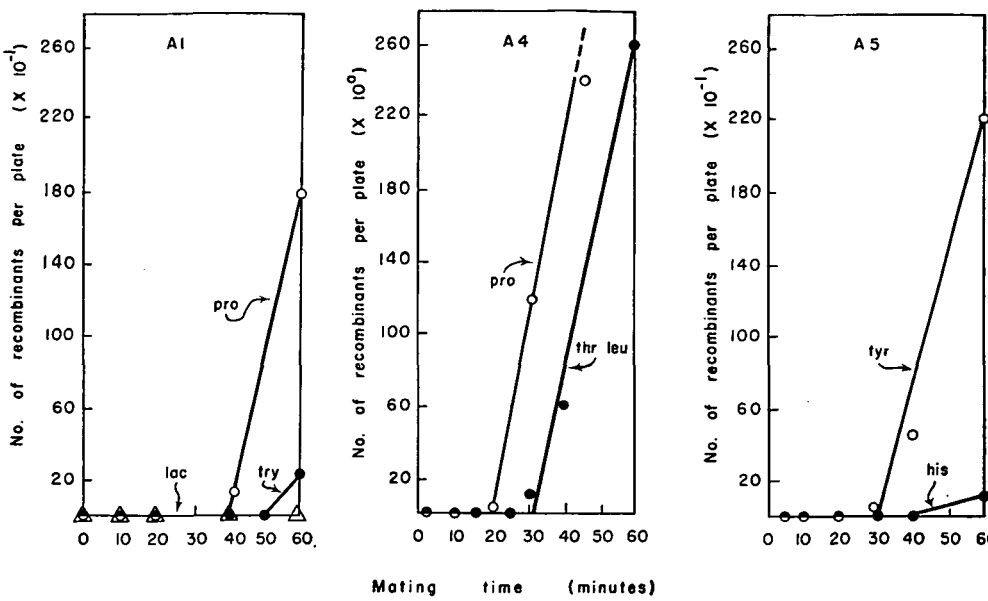

Fig. 2. Chromosome transfer by Hfrs A1, A4 and A5. Crosses were performed by mixing equal volumes of young broth cultures of donor and recipient strains in Erlenmeyer flasks at 37°C. Samples were taken at intervals, diluted and vibrated on a 'Whirlimix' mixer to separate mating pairs before plating on minimal medium appropriately supplemented for the selection of the different (S^r) recombinant classes. Hfr A1 was mated to J62 *pro*$^-$*try*$^-$*his*$^-$$S^r$. Hfr A4 was mated in parallel crosses to W945 *thr*$^-$*leu*$^-$$S^r$ and J62. Hfr A5 was mated against X139 *pyrD his*$^-$*tyr*$^-$$S^r$.

that they all transfer chromosomal markers with a definite orientation, indicating that in each strain the F13 factor is situated at a fixed site. Moreover, each donor strain transfers its markers to the recipient strain in a different order, implying a different location in the chromosome for the sex factor in each case. This result provides strong evidence for a deletion of the *ade-lac* region from the W3747 chromosome; if the region were present, integration would occur largely at this site due to its genetic homology with the chromosomal fragment of the F13 factor (Cuzin & Jacob, 1964; Pittard & Ramakrishnan, 1964).

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The observation (Fig. 2) that the *lac*$^+$ marker of Hfr A1 is missing from its normal position between *pro*$^+$ and *try*$^+$, being transferred much later than either of these markers, demonstrates that at least this part of the *ade-lac* region is deleted from the chromosome.

The orientation of chromosome transfer by the three donor strains is summarized in Fig. 3. It will be noted that *ade*$^+$ is transferred as an early marker by all three

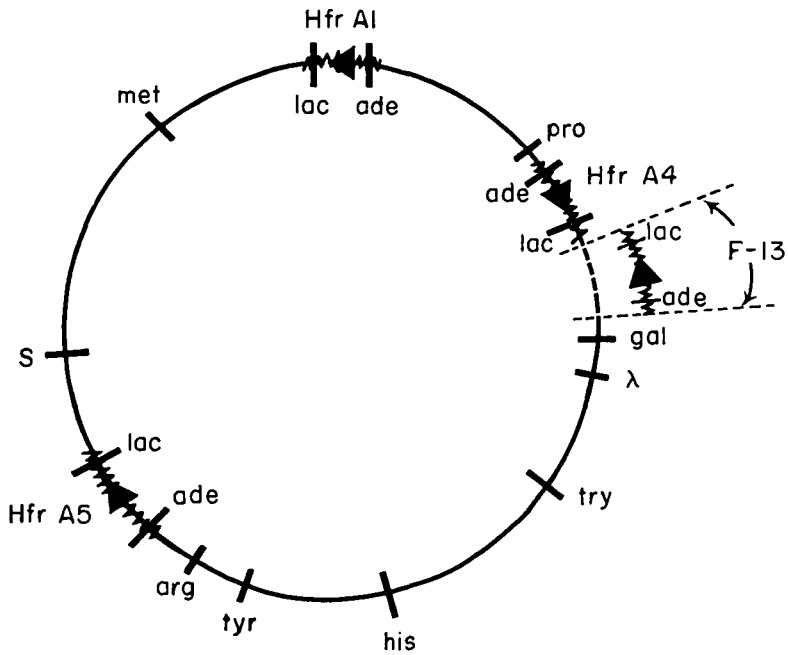

Fig. 3. The location of F13 in Hfrs A1, A4 and A5. The diagram indicates the orientation of the markers transferred by the three Hfr strains in interrupted mating experiments (see Fig. 2). In addition the transfer of *ade*$^+$ and *lac*$^+$ by each donor is shown. This was concluded from similar crosses against X33 F$^-$*ade*$^-$*pro*$^-$*try*$^-$*his*$^-$*S*^{r}, in which there was concomitant selection for *pro*$^+$*S*^{r} (Hfrs 1 and 4) or *his*$^+$*S*^{r} (Hfr 5) to control the time of entry of *ade*$^+$. Markers were concluded to be on the terminal segment of an Hfr donor if they entered recipient cells later than 60 sec. after the onset of mating. Transfer of the prophage λ was measured by mating a donor culture (λ$^+$) treated with λ-antiserum against a λ-resistant, λ$^-$ strain (P678, Jacob & Wollman, 1956). Samples were mixed with a non-lysogenic indicator strain (C600 *S*^{r}) and plated in soft agar on nutrient medium with streptomycin to observe plaque formation by the zygotically induced prophage. F13 is shown in the diagram as a wavy line. The deletion in W3747 is shown as a broken line.

strains, implying that the segment transferred proximally by these strains is not of chromosomal origin, but is the part of the chromosomal segment of the F13 factor carrying *ade*$^+$.

(ii) *The growth of the strain W3747 in AO broth*

Evidence for the loss of a further function from the *ade-lac* region of the W3747 chromosome has been obtained by studying the growth of this strain in AO broth.

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The deletion of a gene essential for cell viability from the W3747 chromosome would explain the isolation of Hfr strains from W3747 in acridine orange. Loss of this function should also lead to the formation of an inviable fraction of cells, lacking the F13 factor, during growth in AO broth. Results confirming this prediction are given in Fig. 4. Curve 1 shows the increase in total cell count of a culture

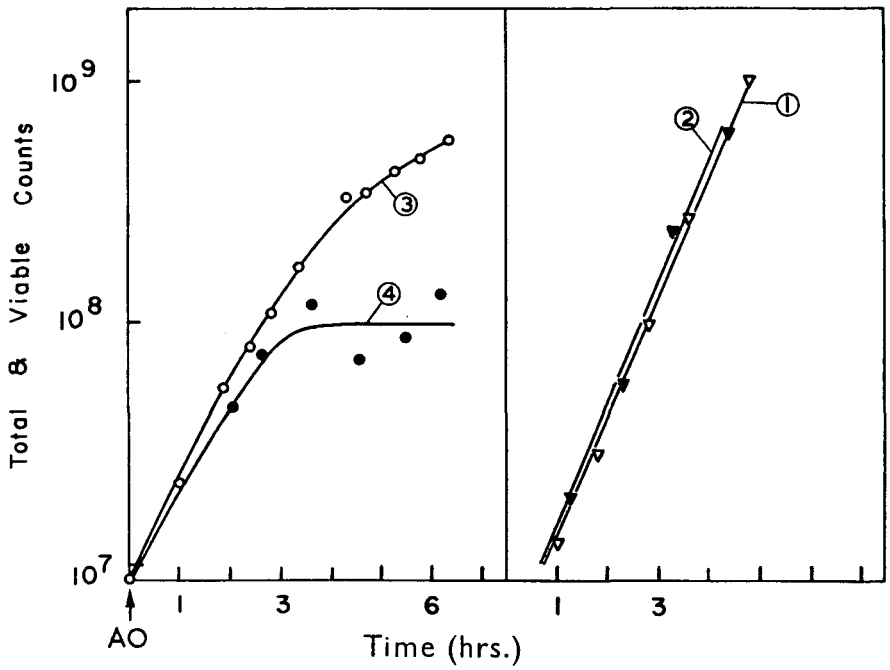

Fig. 4. Growth of the strain W3747 in the presence of acridine orange. A young broth culture of the strain W3747 was diluted into two Erlenmeyer flasks containing nutrient broth, one of which was supplemented with acridine orange (75 γ/ml.). The two cultures were shaken in a reciprocating water bath at 37°C. and at intervals samples were assayed for viable and total cell counts. Samples (·05 ml.) were diluted into 25 ml. of particle-free saline and assayed on a Coulter counter for total cell count. The viable count was assayed on nutrient agar plates. (1) Total counts without AO. (2) Viable counts without AO. (3) Total counts with AO. (4) Viable counts with AO.

growing in broth, for which the viable count (curve 2) was assayed at intervals by plating appropriately diluted samples on nutrient agar. Curves 3 and 4 represent respectively total and viable cell counts obtained for a similar culture growing in broth containing acridine orange (75 γ/ml.). It will be seen that the viable and total cell counts of the control culture increased exponentially and are the same throughout the experiment. The culture containing acridine orange, on the other hand, after an initial period of exponential growth corresponding to about three generations, enters a phase in which the number of viable cells remains constant whilst the total cell number continues to rise. The viable cells must, therefore, be giving rise in AO broth to cells unable to form colonies on transfer to solid medium.

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Such cells were not formed when an Hfr derivative of W3747 (Hfr A5 above) was grown under the same conditions. It is concluded that the inviable fraction formed by W3747 is due to loss of the F13 factor, implying that the F-prime factor carries a chromosomal gene essential for the survival of strain W3747.

4. DISCUSSION

The data presented in this paper show that the strain W3747 has a deletion covering the *ade-lac* region of its chromosome. The consequent absence of gross homology between the chromosomal fragment of the F-prime factor and the W3747 chromosome prevents integration at this site (Cuzin & Jacob, 1964; Pittard & Ramakrishnan, 1964; Scaife & Pekhov, 1964). Rare Hfr strains are thus revealed in which integration has occurred in the absence of extensive homology between the two structures and which can be selected by growth in AO broth.

It seems most likely that the selection thus applied is due to loss of one or more essential metabolic functions concomitant with the removal of the F13 factor from the strain.

Since inhibition of sex factor multiplication by acridine orange is probably very rapid (Stouthamer, de Haan & Bulten, 1963), the initial period of normal growth in AO broth shown by W3747 (Fig. 4) is attributed to the presence of more than one sex factor per cell. After this initial period the total cell count continues to rise, at a (decreasing) rate which may be entirely attributed to the unilinear inheritance of the F13 factor, whose multiplication is assumed to be completely inhibited. Thus the kinetics of growth suggest that, unlike the newly formed daughters, cells still harbouring the sex factor are making enough of the essential gene product to grow. It is concluded that, although F-prime multiplication is inhibited by acridine orange, at least a part of the genetic information on the factor continues to be expressed.

The results presented (Figs. 2 and 3) demonstrate that ade^+ and lac^+, transferred respectively as proximal and distal markers by all three of the Hfr strains investigated, both derive from the F13 factor. The separation of these genes, transferred close together on the autonomous F13 factor (Hirota & Sneath, 1961) is best explained by assuming that integration requires a reciprocal genetic exchange between the region of the chromosomal fragment which separates them and the site on the bacterial chromosome (Fig. 1B). The donor strain resulting from such an exchange should transfer $T6^r$ prior to the ade^+ marker as observed in the autonomous F13 factor (Broda & Hayes, in preparation). Consistent with this prediction is the observation (Table 1) that a significant proportion of ade^+S^r recombinants (which are all F^-) do inherit $T6^r$ from the donor Hfr A1 and that this fraction does not increase with increasing time of mating. A genetic exchange has thus occurred between regions of bacterial chromosome whose homology is concluded from the rarity of integration (Scaife & Pekhov, 1964) to be either short or imperfect. This observation strongly supports the model for F-prime factor formation, which assumes that only limited homology is required for a genetic exchange to occur.

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Table 1. *The nature of* ade$^+$ *transfer of Hfr A1*

Mating time (sec.)	No. of recombinants tested	No. *lac*$^+$	No. *T6*^{r}	No. F$^-$
6	23	0	4	23
60	23	0	3	23

Ade$^+$*S*^{r} recombinants recovered after mating Hfr A1 against X33 F$^-$*T6*^{s}*ade*$^-$*pro*$^-$*try*$^-$*ura*$^-$*try*$^-$*his*$^-$*S*^{r} were purified by restreaking on selective medium before streaking on EMB lactose medium (for the *lac* property) against phage T6 (for the T6 property). In parallel, cultures of the purified recombinants were crossed with W945 *thr*$^-$*leu*$^-$B$_1^-$ *S*^{r} to test for the ability to donate the *thr*$^+$ and *leu*$^+$ markers as a criterion for the acquisition of the donor property together with the *ade*$^+$ marker.

SUMMARY

A model for F-prime formation is presented. It predicts that an Hfr strain giving rise to an F-prime factor would acquire a deletion corresponding to the chromosomal fragment carried by the episome. Genetic studies have confirmed this prediction. Concomitant transfer to the episome of a gene determining a function vital to the cell has permitted selection of derived Hfr strains in which the episomal fragment has been translocated to various sites on the bacterial chromosome.

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