Genet. Res. Camb. (1963), **4**, *pp.* 328–331
With 1 *text-figure*
Printed in Great Britain

The mechanism of chromosome mobilization by an F-prime factor in *Escherichia coli* K12

By JOHN SCAIFE and JULIAN D. GROSS

*Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, London W.*12

(*Received* 24 *April* 1963)

The donor property in *E. coli* is determined by the sex factor, F. In F^+ donors the sex factor exists in an autonomous state and is transferred to recipient cells at a high frequency by infection (Lederberg *et al.*, 1952; Hayes, 1953). Occasionally it may become stably attached to the bacterial chromosome giving rise to Hfr donors which transfer the chromosome in a linear, oriented fashion with the sex factor at the terminal extremity. Autonomous sex factors incorporating the terminal segment of the bacterial chromosome have been isolated from Hfr donors. They are termed F-prime factors. A donor harbouring an F-prime factor transfers its chromosome at a high frequency and with the same orientation as the parental Hfr strain (Adelberg & Burns, 1960; Jacob & Adelberg, 1959). It has been assumed that the first chromosomal marker to be transferred likewise corresponds with that of the parental Hfr.

The F-prime used in our experiments is an F-lac factor. It arose in an Hfr strain transferring as its leading locus the genetic determinant for proline synthesis (*pro*) and as its terminal locus the genes for lactose utilization (*lac*) (Dr. F. Jacob, personal communication). An analysis of recombinants has shown that the F-lac donor, unlike the Hfr, transfers lac as its leading locus.

METHODS AND MATERIALS

Bacterial strains. Two donor strains were used. They were isolated from the same recipient strain after infection with different F-lac factors. One of them harbours an F-lac factor bearing the lac_1 mutation of the strain W945 (Cavalli-Sforza & Jinks, 1956). The

Table 1. *The donor properties of* W1485lac$^+$(*F-lac*$^-$)

Fraction of mated recipient cells which are		Fraction of *pro*$^+S^r$ recombinants which are *lac*$^+$	Fraction of *lac*$^+S^r$ recombinants which are *pro*$^+$
pro$^+$	*lac*$^+$		
2·5%	2·2%	15%	31%

One millilitre of a young broth culture of W1485*pro*$^+$*lac*$^+S^s$(F-lac$^-$) was mixed with 1 ml. of an overnight culture of W945*pro*$^-$*lac*$^-S^r$. After gentle aeration for 60 minutes the mixture was diluted, agitated violently to stop further transfer and aliquots were then spread on appropriately supplemented plates to select for *pro*$^+S^r$ and *lac*$^+S^r$ recombinants. The recombinants obtained were scored for the unselected character. The total number of mated cells was estimated by measuring the number of recipient cells which became *lac*$^+$ in a parallel experiment mating the same recipient strain with the W1485*pro*$^+$*lac*$^+S^s$(F-lac$^+$) donor.

other carries an F-lac$^+$ factor. Both strains have the chromosomal genotype W1485-*met*$^-$*pro*$^+$*lac*$^+$*S*^{s}.

The recipient strain used was obtained by recombination from the strain W945. It has the genotype W945*pro*$^-$*B*$_1$$^-$*lac*$_1$*S*^{r}.

Media and culture methods were as described by de Haan & Gross (1962). Abbreviations are given under Table 1.

RESULTS

The strain W1485*lac*$^+$(F-lac$^+$) behaves as a typical F-prime donor. It transfers *lac*$^+$ at a high rate by F-lac infection and *pro*$^+$, forty to a hundred times less frequently, as a chromosomal marker. The strain W1485*lac*$^+$(F-lac$^-$), on the other hand, cannot transfer *lac*$^+$ at a high rate by infection since its F-lac factor has the *lac*$^-$ genotype. It is this strain which has been used to demonstrate linked transfer of the chromosomal markers *lac*$^+$ and *pro*$^+$ by an F-lac donor.

The strain W1485*lac*$^+$(F-lac$^-$) was mated with a *pro*$^-$*lac*$^-$*S*^{r} recipient and fifty of the *pro*$^+$*S*^{r} and *lac*$^+$*S*^{r} colonies recovered were then streaked on appropriate media to determine the number of *lac*$^+$ amongst the former and the number of *pro*$^+$ amongst the latter class. The results are shown in Table 1. It can be seen that 15% of the *pro*$^+$*S*^{r} recombinants had inherited *lac*$^+$, as against only 2.2% in the total mated population, and that nearly one-third of the *lac*$^+$*S*^{r} recombinants had received *pro*$^+$.

Table 2. *Chromosomal transfer of* lac$^+$ *by W*1485lac$^+$(*F-lac*$^-$)

Mating time (min.)	No. of *pro*$^+$*S*^{r} recombinants per plate	Fraction of *lac*$^+$ amongst *pro*$^+$*S*^{r} recombinants	
		No. tested	*lac*$^+$(%)
10	487	308	14·9
20	910	303	13·6
40	2205	299	17·1

A young broth culture of W1485*lac*$^+$(F-lac$^-$) (diluted 1 in 20 in broth) was mixed with an equal volume of an overnight culture of W945*pro*$^-$*lac*$^-$*S*^{r} at 37°C and gently aerated. Samples were removed at intervals and violently agitated to separate mating pairs, before plating on supplemented minimal medium to select *pro*$^+$*S*^{r} colonies. These were subsequently streaked on EMB lactose medium to determine the fraction of *lac*$^+$.

These results suggest that F-lac donor cells are able to transfer *lac*$^+$ as a chromosomal marker linked to *pro*$^+$. This conclusion is borne out by the following findings. Firstly, the *lac*$^+$ phenotype of most of the *lac*$^+$*pro*$^+$*S*^{r} recombinant bacteria has been shown to be stable to the action of acridine orange, which removes autonomous F-prime factors (Hirota & Sneath, 1961; Scaife & Gross, 1962). The second finding is reported in Table 2. This shows the result of an interrupted mating experiment using the W1485*lac*$^+$(F-lac$^-$) donor. It is found that the fraction of *pro*$^+$ recombinants which are *lac*$^+$ does not increase with increasing time of mating, indicating that *lac*$^+$ is being transferred at the leading extremity of the donor chromosome proximal to the *pro*$^+$ marker selected in the cross.

DISCUSSION

Figure 1 presents the model for the mechanism of chromosome mobilization by F-prime factors which has led to the experiments reported above. It rests on two assumptions. The first is that the F-lac factor itself is transferred as an oriented linear structure. The second is that the initial stage of chromosome mobilization is the synapsis of the F-lac

factor with the homologous *lac* region of the circular donor chromosome. It is proposed that a reciprocal genetic exchange occurs between the F-lac factor and the chromosome within the region of pairing. The two now form a single oriented structure which is linear in transfer, carries a terminally attached sex factor and possesses the lac segment in duplicate (Fig. 1 (3)).

It may be seen that, depending on the position of the genetic exchange, the resulting structure has either the *lac*$^+$ or the *lac*$^-$ marker at its leading extremity. In the present communication we have presented evidence for the existence of the former class of donor cells, *viz.* those transferring *lac*$^+$ as the leading locus. Evidence for the existence of the other class of donor will be presented in a later publication, along with additional experi-

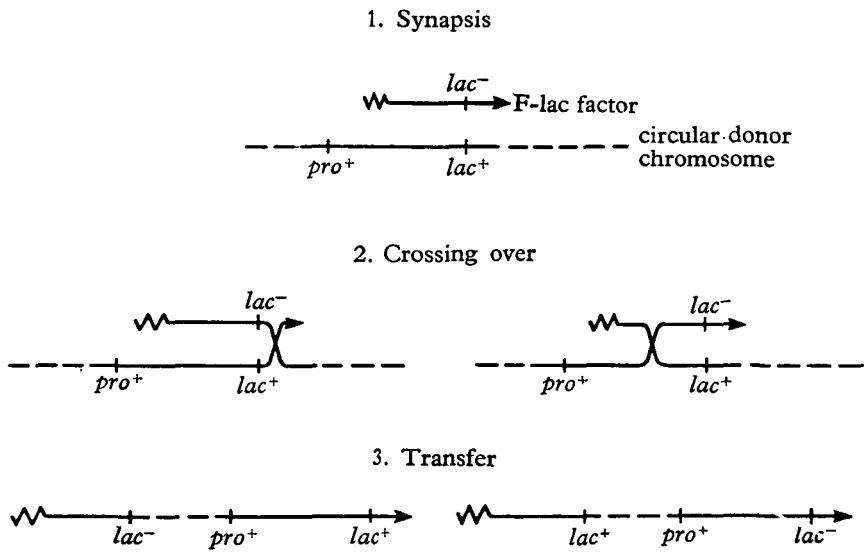

Fig. 1. Chromosome mobilization in an F-lac donor. The arrow represents the leading extremity of the genetic structure transferred during conjugation, while the wavy line represents the genetic material of the sex factor, F. *Note*: The F-lac factor, drawn here as a linear structure, is formally equivalent to a circle opening at a specific point prior to transfer.

mental support for the proposed model. It may be noted here that the rather low frequency of inheritance of *lac*$^+$ by *pro*$^+$ recombinants in the experiment reported in Table 1 indicates that donors transferring *lac*$^+$ at the leading extremity are considerably less frequent than those transferring *lac*$^-$. This inference, which is easily accounted for if the *lac*$^-$ marker is situated towards the right-hand end of the F-lac factor (Fig.1 (2)), is confirmed by observations to be reported subsequently. Such a dependence of the ratio of the two types of donor on the position of the marker on the F-prime factor may also be clearly inferred from recent observations of Pittard *et al.* (1963). These workers have shown in addition that transfer of chromosomal markers by F-prime strains is delayed compared with transfer of the same markers by the parental Hfr strain. In agreement with the model presented here, they have observed that the extent of the delay corresponds to the time required to transfer the segment of genetic material incorporated in the F-prime factor.

REFERENCES

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

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

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

HAYES, W. (1953). The mechanism of genetic recombination in *E. coli*. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 75–93.

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

JACOB, F. & ADELBERG, E. A. (1959). Transfert de charactères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. *C. R. Acad. Sci.* (Paris), **249**, 189–191.

PITTARD, J., LOUTIT, J. S. & ADELBERG, E. A. (1963). Gene transfer by F′ strains of *Escherichia coli* K12. I. Delay in initiation of chromosome transfer. *J. Bact.* (In press).

PITTARD, J. & ADELBERG, E. A. (1963). Gene transfer by F′ strains of *Escherichia coli* K12. II. Interaction between F-merogenote and chromosome during transfer. *J. Bact.* (In press).

LEDERBERG, J., CAVALLI, L. L. & LEDERBERG, E. M. (1952). Sex compatibility in *E. coli*. *Genetics* **37**, 720–730.

SCAIFE, J. & GROSS, J. D. (1962). Inhibition of multiplication of an F-lac factor in Hfr cells of *Escherichia coli* K12. *Biochem. biophys. Res. Comm.* **7**, 403–407.