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SHORT NOTES

Low recombination frequency for markers very near the origin in conjugation in *E. coli**

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Conjugation in *E. coli* involves linear transfer of Hfr (male) chromosomal material into a recipient cell. The sequence of transfer of the genetic markers is determined by the particular Hfr strain used; the closed *E. coli* linkage group is opened at a point (origin) particular to the given Hfr strain, and markers are transferred in a polarized order from this point. In general, markers that are located far from the origin appear in recombinants with a low frequency, due to interruption of transfer at randomly distributed points along the linkage group (Jacob & Wollman, 1961, pp. 144–154). This decrease in the number of recombinants with increasing distance of the marker from the origin is called the transfer gradient. The data reported below show that markers located very close to the origin (i.e. transferred very early in conjugation) appear in recombinants less often than expected from an extrapolation of the transfer gradient. The results are consistent with the requirement that for a male marker to appear in a viable recombination at least one cross-over between male and female chromosomal material must occur at some point at random between the origin and that male marker. The average cross-over frequency may be of the same magnitude as observed for cross-overs between markers located elsewhere on the chromosome.

MATERIALS AND METHODS

The strains used in these experiments are listed in the first column of Table 1. The second column shows the entry times for the Hfr wild-type characters used, as determined from interrupted mating experiments using the female strains which carried the corresponding auxotrophic alleles. (Symbols for nutritional requirements: arg—arginine, thr—threonine, leu—leucine, pro—proline, lys—lysine, his—histidine.) Streptomycin was used to counterselect against the males in all cases except for AB 313 × KL63, where leucine starvation was used for counterselection.

Hfr strains H (Hayes), C (Cavalli) and 1 were donated by Dr A. Garen; Hfr strains AB261 and AB313 were donated by Dr A. L. Taylor; Hfr strains Ra2 and KL16 were derived in this laboratory from F^+ strains.

Matings were accomplished by mixing exponentially growing broth cultures (10^8 cells per ml.) of male and female cells in a 1:10 cell ratio and gently agitating these for

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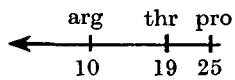
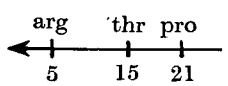
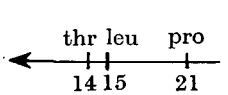
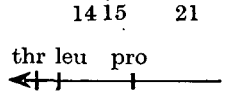
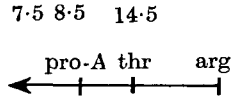
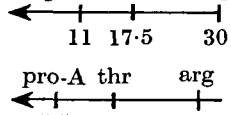
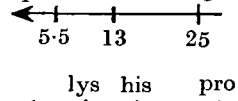
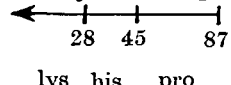
90 min. at 37°C. Aliquots were then diluted and spread onto recombinant-selective plates. The growth media have been previously described (Marcovich, 1961). Interrupted matings were performed using a vibratory blending device (Low & Wood, 1965). All experiments were done at least three times to insure reproducibility.

Table 1. *Analysis of matings of various* E. coli *strains*

E. coli K-12 strains	Male marker entry times (minutes)	Recombination ratio for two distal markers	Recombination ratio of marker near origin to distal marker	Relative number of recombinants with marker near origin	Calculated entry time for origin (minutes)
Hfr 1 × AB1133		$\frac{thr}{pro} = 1{\cdot}7$	$\frac{arg}{thr} = 1{\cdot}8$	(1·00)	—
Hfr Ra2 × AB1133		$\frac{thr}{pro} = 1{\cdot}6$	$\frac{arg}{thr} = 0{\cdot}43$	(arg) 0·24	4·3
Hfr Ra2 × PA330		$\frac{leu}{pro} = 2{\cdot}4$	$\frac{thr}{pro} = 2{\cdot}2$	(1·00)	—
Hfr H × PA330		$\frac{leu}{pro} = 2{\cdot}2$	$\frac{thr}{pro} = 1{\cdot}4$	(thr) 0·63	5·0
Hfr C × AB1133		$\frac{thr}{arg} = 2{\cdot}9$	$\frac{pro\text{-}A}{thr} = 2{\cdot}7$	(1·00)	—
Hfr AB261 × AB1133		$\frac{thr}{arg} = 3{\cdot}0$	$\frac{pro\text{-}A}{thr} = 1\ 8$	(pro) 0·69	2·6
Hfr AB313 × KL63		$\frac{his}{pro} = 13$	$\frac{lys}{his} = 4{\cdot}1$	(1·00)	—
Hfr KL16 × KL63		$\frac{his}{pro} = 14$	$\frac{lys}{his} = 0{\cdot}37$	(lys) 0·09)	4·8

RESULTS

Various crosses were carried out, with the general purpose of comparing the recombination frequency of a male marker very near the origin in one Hfr strain with the recombination frequency of the same marker transferred later by another Hfr strain. Recombination frequencies for two additional markers transferred later (more distally) by each male were also determined, thereby allowing proper correction to be made for effects other than the one under study.

Results from matings of four pairs of Hfr strains with female strains are given in Table 1. The first two pairs transfer markers in the 'clockwise' direction as related to published genetic maps; the second two pairs transfer in the 'counterclockwise' direction. The Hfr's of a given pair were mated in parallel experiments with the same female strain. The two Hfr strains in a given pair differ only in that their origins are located at slightly

different locations on the chromosome. Hence, for each pair there is a marker which is transferred very early by one Hfr strain and somewhat later by the other.

From the second column of Table 1 it is apparent that the intervals between marker entry times are approximately the same for each Hfr strain in a given pair, even though the origins are not the same, as mentioned before. The third column shows the ratios of numbers of recombinants for the two more distal markers for each pair of Hfr strains. Within each pair of strains these ratios are seen to be essentially constant, indicating that the transfer gradients generated by more distal markers are the same for each member of the pair.

The fourth column gives the ratios of the number of recombinants having the early male marker to the number having one of the distal markers. When the first of the three markers is very near the origin, it appears in each case in substantially fewer recombinants (relative to the numbers of recombinants having the distal markers) than when it is transferred later, i.e., not so close to the origin. The relative number of recombinants from the Hfr with the marker near the origin compared to the number from the other Hfr strain of the pair, using the numbers from column four, is given in the fifth column.

DISCUSSION

The fifth column of Table 1 shows that in all four Hfr pairs, a marker transferred very early appears in recombinants with a lower frequency than when transferred later. Also, the amount of 'depression' of the very early marker differs from case to case. The amount of depression is greatest for the markers with the earliest entry times.

Of relevance here is the question of when the first bit of Hfr genetic material (i.e., the origin) is transferred to the female. Among the Hfr strains listed, and with all others studied in this laboratory, none has been found to transfer a marker earlier than approximately 5 min. after mixing male and female cells together. A lower bound can also be put on the origin entry time by using an Hfr strain with an origin located between two markers located close together on the closed linkage group (genetic map) such that one of the two markers is transferred very early and the other is transferred very late (distally). Therefore the physical distance between the origin and the earlier marker is at most given by the short chromosomal interval between the two markers on the genetic map. Thus, Hfr AB261 transfers the proline marker pro-A to recipient AB1133 at 5·5 min., and transfers distally another marker pro-B which is separated from Pro-A by 2 min. on the closed linkage group (Taylor & Thoman, 1964). This indicates that the first chromosomal marker from Hfr AB261 has an entry time of not less than 3·5 min. Similarly, Hfr KL16 transfers the lysine marker at 5 min. and transfers distally serine-A, which is located 2 min. away on the genetic map; therefore the first chromosomal marker from Hfr KL16 has an entry time of not less than 3 min. From these accumulated data we can estimate the origin entry time at between 3 and 5 min. after mixing male and female cells. (This applies only to those mating pairs in the mating suspension which are the earliest to begin transfer. For other mating pairs there are further delays in transfer which may be related to the various physiological ages of the cells (de Haan & Gross, 1962; Jacob, Brenner & Cuzin, 1963).)

Nearly all zygotes contain only a partial male genome and in order to form a viable recombinant they must use female genetic material adjacent to the origin in the distal region. Therefore a reasonable explanation for the observed depression of frequency of appearance of very early male markers is that in the merozygote there must be at least one random cross-over between male and female chromosomal material between the origin and any male marker in order for that male marker to appear in a viable recombinant. Increased depression for male markers closer to the origin follows due to smaller

probability for cross-overs within smaller chromosomal segments. One would not expect an early marker depression effect if the necessary cross-over near (or at) the origin were not a random event but were somehow automatically accomplished whenever recombination occurs.

If we assume that the cross-over frequency near the origin is the same as that along the rest of the chromosome, it is possible to estimate the origin entry times from the amount of depression of the early marker as given in Table 1. Jacob & Wollman (1961, p. 234) reported approximately 20 recombination units per minute of chromosomal length (in units of transfer time) for the average cross-over frequency along the chromosome during recombination. Another estimate of the cross-over frequency can be made by analyzing the recombinants from the matings of Table 1 in order to find the average threonine–leucine linkage. The observed linkage is 87%. Presently available data show that the entry time interval between threonine and leucine is between $\frac{1}{2}$ and 1 min. in length (Taylor & Thoman, 1964). These limits indicate a cross-over frequency of between 13 and 26 recombination units per minute.

In order to relate the recombination frequency of a very early male marker with the distance of the marker from the origin, the effects of both the transfer gradient and the cross-over probability must be considered. The transfer gradient is an exponential decrease in the number of zygotes receiving a given male marker as a function of the distance of the marker from the origin. As long as a marker is not close enough to the origin to be depressed due to a lack of cross-overs, the ratio of its frequency of appearance in recombinants to that of a more distal marker is independent of the position of the origin (Table 1, column 3). A decrease in this ratio, in a case where the origin is close to the earlier marker is due, we assume, to a low probability of cross-overs in the short interval between the origin and the marker. This is related to the classical linkage of any two closely spaced markers on separate but homologous genomes, compared to the linkage of two analogous widely spaced markers. The relative frequency of appearance of a marker near the origin due to cross-overs alone is given by $(1-e^{-2Kx})$ (see Stahl, 1964), where x is the distance between the origin and the male marker, and K is the cross-over probability per unit length of chromosome. With $K=0{\cdot}2$/min. from the data discussed above, $(1-e^{-2Kx})$ rapidly approaches 1·0 as x increases, and differs from 1·0 by less than 10% for x greater than about 5 min. If we use Table 1, fifth column, for the relative frequencies of appearance for markers near the origin we can extract the values of x for the various cases studied. By subtracting x from the marker entry time, the calculated origin entry time is obtained and is listed in the last column of Table 1. These origin entry times are generally compatible with the 3–5 min. interval estimated earlier, above, from genetic map data and early marker entry time data. Presumably, the origin entry time depends somewhat on the particular strains used; by mating a given male strain with different female strains, the early male marker entry times have been found to vary by as much as $1\frac{1}{2}$ min.

The present experiments do not indicate what occurs during the 3–5 min. interval preceding transfer of the earliest chromosomal marker from male to female cell. A part of this time may involve transfer of part of the sex factor which is linked to the male chromosome at the location of the origin. The other portion of the 3–5 min. interval, preceding transfer of any type of genetic material, is presumably devoted to construction of a transfer tube and/or 'mobilization' of the male genetic material prior to transfer.

The data and analysis above indicate that male markers transferred more than 4–5 min. after the origin (i.e., having entry times later than 8–9 min.) will not show any appreciable depression, due to the rapid fall-off of the effect with increasing distance from the origin. On the other hand, markers very close to the origin are significantly depressed, such as the lysine marker in the cross KL16 × KL63, where it appears with less than 10% of the frequency expected from the transfer gradient alone.

SUMMARY

By performing matings using various Hfr strains having different origins of transfer, it has been observed that male chromosomal markers located very near the origin of transfer in conjugation in *E. coli* appear in recombinants with a lower frequency than expected from an extrapolation of the transfer gradient. The effect is greatest for markers closest to the origin, and becomes negligible for markers transferred more than 4–5 min. after the origin. The results suggest that in the zygote a random cross-over between male and female genomes is necessary somewhere between the origin and any male marker in order for that marker to appear in a viable recombinant. It was also deduced that the entry time for the origin itself occurs after a 3–5 min. delay after mixing male and female cells together.

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