EVIDENCE FOR A CLOSED LINKAGE GROUP IN HFR MALES OF ESCHERICHIA COLI K-12[1]

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The fertility of populations of F^+ strains of Escherichia coli is due in large part to the presence of rare Hfr mutants which effect a progressive, unilateral transfer of linear chromosome segments to F^-, or female, recipient cells (Jacob & Wollman, 1956). The circular chromosome model of Jacob and Wollman (1957) was advanced to account for the diverse Hfr types present in F^+ populations. In the mutation $F^+ \longrightarrow$ Hfr, the autonomous sex factor, F, becomes stably attached to the F^+ chromosome at any of several sites; this event severs the closed linkage group to yield a linear structure with F attached to one extremity, the other free end being termed the Origin. Origin is the first portion of the chromosome to penetrate the female cell during conjugation.

The closed structure of the F^+ chromosome has been amply verified by linkage analyses with a variety of Hfr strains (Jacob & Wollman, 1958; Taylor & Adelberg, 1960). Wollman and Jacob (1959) state that linkage between proximal and distal Hfr markers is restored in those progeny of an Hfr X F^- cross which have received the extreme distal markers of the male parent. This suggests that the F^- chromosome is also a closed structure. Hfr strains are

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thus currently considered to have rectilinear chromosomes, whereas F^+ and F^- strains are believed to possess closed, or circular chromosomes.

This paper presents genetic evidence to suggest that the chromosomes of non-donor Hfr cells exist as closed, rather than linear, structures.

Materials and Methods. Media and techniques for the selection and scoring of genetic recombinants have been previously described by Adelberg and Burns (1960). Mating mixtures contained a minority parent at a cell density of 2×10^7 cells/ml, and a majority parent at 4×10^8 cells/ml. The mixtures were sampled and plated for recombinant selection after 120 minutes of undisturbed incubation at 37 C. Samples were washed once with buffer before plating. F^- recipient phenocopies of Hfr and F^+ strains were prepared by subjecting stationary phase broth cultures to vigorous aeration at 37 C for a period of 6 to 8 hours.

Bacterial strains are listed in Table I. Strains AB-1340, 1342, and 1348 are all recombinant males derived from a single Hfr X F^- cross; it follows that they possess identical points of Origin. They transfer the sequence Origin-Tl-pro-his-xyl-met-F.

Table I

Bacterial Strains

Strain no.	Genetic markers					Mating type
	phage Tl	pro	his	xyl	met	
AB-1340	S	+	+	-	-	Hfr
AB-1348	R	-	-	+	+	Hfr
AB-1342	S	-	+	-	-	Hfr
AB-1352	S	+	+	+	-	F^+

Abbreviations: pro, his, met, nutritional requirements for proline, histidine and methionine, respectively; xyl, utilization of D-xylose as energy source; S, sensitive; R, resistant.

Results. Aerated, stationary phase cultures of F^+ males undergo a reversible phenotypic change to the F^- state. These F^- phenocopies are no longer fertile as males, but may now serve as genetic recipients (Lederberg, Cavalli, & Lederberg, 1952). Hfr males behave similarly in our hands; the effect is entirely reversible,

with full donor capacity being restored in less than two generations of exponential growth.

The fertility of homosexual Hfr crosses permits a simple linkage test for the presence of closed or linear chromosomes in recipient Hfr cells. Referring to Figure 1, Model 1 predicts that recombinants which receive $\underline{met}^+$ from the donor will inherit the unselected markers $\underline{T1}^r$ or $\underline{pro}^-$ at frequencies no greater than are encountered in recombinants selected for a more proximal marker such as $\underline{xyl}^+$. Model 2, on the other hand, predicts that met and T1 will be linked; that is, $\underline{met}^+$ recombinants will inherit $\underline{T1}^r$ or $\underline{pro}^-$ at a higher frequency than $\underline{xyl}^+$ recombinants.

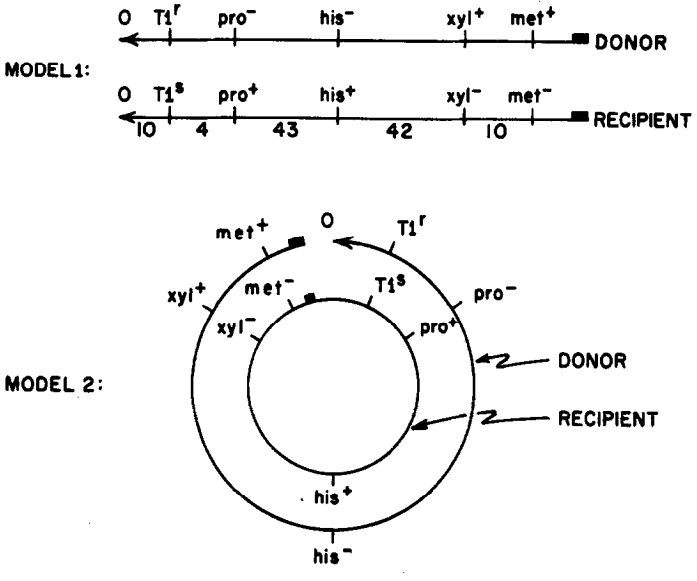

Figure 1. Diagrammatic representation of complete zygotes for recipient cells possessing a linear (Model 1) or a closed (Model 2) chromosome. Numbers refer to interlocus map distances in minutes. The symbols O and ■ represent Origin and attached sex factor, respectively.

The experiment depicted in Figure 1 was performed by mating strain AB-1348 with a phenocopy F^- culture of strain AB-1340. The data presented in Table II, cross 1, clearly indicate that the proximal donor markers, $\underline{T1}^r$ and $\underline{pro}^-$, are linked to the terminal marker, $\underline{met}^+$. By contrast, selection for $\underline{his}^+$ in the reciprocal cross of AB-1340 X phenocopy F^- AB-1348 results in a much lowered frequency of inheritance of the proximal donor markers (Table II, cross 2).

Table II

Bacterial Crosses

Cross no.	Donor parent	Recipient parent: phenocopy F^-	Selected donor marker	Recombination frequency	Frequency of unselected donor markers in percent T1	pro	No. of colonies scored
1	AB-1348	AB-1340	$\underline{xyl}^+$ $\underline{met}^+$	0.196 0.060	53 71	31 54	200 400
2	AB-1340	AB-1348	$\underline{his}^+$	0.25	19	22.5	200
3	AB-1348	AB-1342	$\underline{xyl}^+$ $\underline{met}^+$	0.087 0.038	50 69.5	- -	200 200
4	AB-1348	AB-1342$^+$ AB-1352	$\underline{met}^+$	0.031	68	1.5 $\underline{pro}^+$	200

The minority parents are the donors in crosses 1 and 2, and the recipients in crosses 3 and 4. Recombination frequencies are expressed as the number of recombinants recovered per 100 minority parent cells in the mating mixtures. Donor parents were contraselected by omitting histidine from the selective media in crosses 1, 3, 4, and by omitting methionine in cross 2.

All Hfr cultures are contaminated with small numbers of F^+ cells which arise from the backmutation Hfr $\longrightarrow$ F^+. Preferential mating of the donor Hfr with F^+ revertants in the recipient population could potentially account for the linkage data we have presented. This contingency has been examined in the following reconstruction experiment.

Strain AB-1342 was deliberately contaminated, to the extent of 3.5 percent, with phenocopy F^- cells of the F^+ strain AB-1352, and then mated with AB-1348. A cross of AB-1348 X AB-1342 alone served as the control. F^+ revertants were present in the AB-1342 population at a level of approximately 0.1 percent. If preferential mating with the F^+ cells occurs, one expects first that the artificial mixture will yield more $\underline{met}^+$ recombinants than the control, and second, that roughly one half of these recombinants will harbor the differential marker, $\underline{pro}^+$, which distinguishes the F^+ from the two Hfr parents. The results in Table II, crosses 3 and 4, show that neither expectation is fulfilled. In fact, the donors appear to mate at random with both F^+ and Hfr recipients. We conclude that the contribution of F^+ revertants to the linkage data is negligible.

Discussion. Fredericq (1960) has recently reported some experiments which he interprets as showing the Hfr chromosome to be an open, linear structure. Although he does not state the conditions of his cross, or the markers used to contraselect his donor strains, his data are difficult to reconcile with ours. It seems likely, however, that the cells in which he finds a rectilinear chromosome were cells which had initiated the process of chromosome transfer, and were therefore typical of conjugating donor cells in which the chromosome is known to be broken. The restoration of genetic linkage which we have observed between proximal and terminal markers in homosexual Hfr crosses demonstrates, however, that the chromosome of a non-donor Hfr male is a closed structure, similar to the one postulated for F^+ and F^- strains. Hfr males differ, of course, from the latter in possessing the potentiality for converting the closed linkage group into a linear one in order to effect transfer. A corollary of this finding is that the stable attachment of F to the bacterial genome does not mandatorily determine a break in the structure, but rather determines the point at which rupture will occur in a donor cell at the time of conjugation. The preservation of the intact, closed genome in non-donor Hfr cells not only presents a uniform picture for the chromosomal structure of E. coli K-12, but also accounts for the viability of a unique "double" Hfr male, recently prepared by Dr. A. J. Clark (1961), which simultaneously harbors two attached F agents.

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