# THE ORIENTATION AND EXTENT OF GENE TRANSFER IN ESCHERICHIA COLI

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*Introduction.*—Genetic recombination of the type first described by Lederberg and Tatum[1] in *Escherichia coli*, strain K12, has been shown to involve conjugation[2] between sexually differentiated cells ($F+$ or *Hfr* with $F-$).[3, 4] Unilateral gene transfer from *Hfr* (high frequency of recombination) to $F-$ cells is suggested by the observation that recombinants occur only among the progeny of the $F-$ exconjugants.[2, 4] In recent experiments by Wollman and Jacob,[5] *Hfr*:$F-$ conjugants were separated by rapid stirring in a Waring Blendor, and then recombinants arising from the transfer and incorporation of two linked *Hfr* genes ($T+$ and $L+$) were selected by plating on appropriate minimal medium. It was found that, among these selected recombinants, the frequencies of several other, unselected *Hfr* genes increased with the duration of conjugation before the parental pairs were separated. These results indicate that all genes are not transferred simultaneously and suggest that, under these conditions, partial genomes may be transferred from *Hfr* to $F-$ cells. Further interpretation, however, is limited by the techniques employed. First, owing to the exclusive use of selected recombinants, the existence of a single, or preferred, sequence of gene transfer is not clear. It could be argued, for example, that during conjugation the probability of multiple gene transfer increases, but that no specific gene is regularly transferred earlier than another. In the present investigation the analysis of unselected recombinants has revealed that gene transfer is indeed oriented. Second, the method of disrupting pairs is sufficiently drastic to raise the possibility that the inferred transfer of partial genomes may not characterize "normal" mating.[6] In the present study this possibility has been minimized by the observation of exconjugants which originate in the absence of artificial disruption.

*Materials.*—Two *Hfr* strains are employed, both ultimately derived from the $F+$ strain, 58-161. W2323 is a subculture of the *Hfr* strain isolated by Hayes;[7] CS101 is a $V_1^r\ V_2^r$ derivative of W1895, the *Hfr* strain originally isolated by Cavalli.[8] CS2 is an $F-$ derivative of 58-161. W2323 and CS2 share the following genotype: $M-\ T+\ L+\ Th+\ Lac_1+\ Mal_1+\ V_1^s\ V_2^s\ V_6^s$; CS101 differs only in being $V_1^r\ V_2^r$. CS100 is an $F-$ strain derived from Y53 and is of the following genotype: $M+\ T-\ L-\ Th-\ Lac_1-\ Mal_1-\ V_1^s\ V_2^s\ V_6^r$. The origin of 58-161 and Y53 is described elsewhere.[9] The designations $M-$, $T-$, $L-$, and $Th-$ refer to methionine, threonine, leucine, and thiamine growth requirements, respectively, $Lac-$ and $Mal-$ to inabilities to ferment lactose and maltose, and $V_1^r$, $V_2^r$, and $V_6^r$ to resistance to coliphages T1 (and T5), T2, and T6. Wild-type T1, T2H, and T6 were employed; T2 and T6 were inactivated by a dose of ultraviolet light which produced about 50 lethal hits per phage.

*Experimental Procedures and Results.*—When preincubated mixtures of CS101 (*Hfr Lac+ Mal+*) and CS100 ($F-$ *Lac− Mal−*) are plated on complete media

which permit the differentiation of parental fermentative types (EMB-lactose or EMB-maltose[10]), a high percentage of colonies are characteristically sectored. These colonies contain both fermenters and nonfermenters and resemble closely in appearance the segregating heterozygotes pictured by Lederberg.[11] Since these variegated colonies (*Lacv* and *Malv*) do not appear when preincubated mixtures of CS2 (*F*− *Lac*+ *Mal*+) and CS100 are similarly plated, they presumably reflect the mating process.

In order to examine the kinetics of formation of those units which give rise to variegated colonies on plating, the following standard procedures were adopted, which also met the demands of concurrent radiochemical studies.[12] Parental cultures, grown for 12 hours in Difco Penassay broth at 37° C. without aeration, were washed and resuspended in peptone broth (1 per cent Difco Bacto-Peptone, 0.3 per cent NaCl, 0.1 per cent glucose, $10^{-3}$ *M* $MgSO_4$, $10^{-4}$ *M* $CaCl_2$) at one-twentieth of their original concentration. After aeration at 37° C. for $2^1/_4$ hours (to give about $2 \times 10^8$ cells/ml), the cultures were centrifuged, washed, and resuspended at a twofold dilution in Penassay broth. After further aeration at 37° C. for 30 minutes, the cultures were centrifuged and concentrated fivefold in fresh Penassay broth (to give about $10^9$ cells/ml). At this time, the cultures were mixed, 1 part *Hfr* to 2 parts *F*−, and aerated at 37° C. Under these conditions, the total cell titer increased about twofold during 80 minutes of mixed growth. After varying intervals, samples were removed from the mixture and either were directly diluted in saline and plated to give isolated colonies on EMB-lactose or EMB-maltose or were exposed to UV-inactivated T2 or T6 prior to dilution and plating. Phage exposure consisted of 5 minutes' adsorption in buffer at a multiplicity of 20 phage/cell and at a cell concentration of $2 \times 10^8$ per milliliter. The numbers of "+" (fermenters), "−" (nonfermenters), and variegated colonies were determined after 18–24 hours' incubation at 37° C. Mutant "+" papillae appeared later on "−" colonies and were easily distinguished from the more sectorial variegation.

At no time did variegated colonies (*Lacv* or *Malv*) appear following exposure to T2 (which attacks the *F*− parent), as is to be expected if gene transfer is unilateral from *Hfr* to *F*−.

Figure 1 shows the results of experiments in which measurements were made simultaneously of the frequencies of *Lacv* and *Malv* colonies before and after exposure to T6 (which attacks the *Hfr* parent) at different times during the mating period. Each point is based upon a sample containing $10^3$–$10^4$ *Hfr* cells as pooled from three separate experiments. The total number of *Hfr* cells at each time was taken as the number of *Mal*+ and *Malv* colonies which appeared when cells were not exposed to T6 before plating. The results are consistent with the following interpretation.

Conjugating pairs yield variegated colonies (*Lacv* and *Malv*) when plated without exposure to T6. Exposure to T6 kills the *Hfr* parent and thus destroys the capacity of a pair to produce a variegated colony unless transfer of the pertinent gene to the *F*− cell has already occurred and will be followed by incorporation. In the latter event, since the surviving *F*− cell is multinucleate, it is still capable of producing a variegated colony. Therefore, *Lac*+ is transferred and incorporated more frequently than *Mal*+, since *Lacv* colonies suffer much less reduction than *Malv* colonies following exposure to T6. In fact, the number of *Malv* colonies after T6

exposure is so small that the number of *Malv* colonies which appear when cells are not exposed to T6 may be taken as a measure of plated pairs. The excess of *Lacv* over *Malv* observed when cells are not exposed to T6 is ascribable to fertilized *F*− cells which have separated from their *Hfr* partners prior to plating. These "spontaneous" exconjugants appear as early as 10 minutes after mixing the parental strains and increase throughout the period studied.

On the preceding interpretation, exposure to T6 removes *Hfr* parental cells and leaves unfertilized *F*− cells together with an unselected array of fertilized cells The latter are sufficiently numerous that their derivative clones may be analyzed by replica-plating.[13] Accordingly, mixtures of CS101 and CS100, after exposure to T6 at various intervals during the mating period, were plated on EMB-lactose (nonselective medium) as before. Then, after scoring for *Lacv*, all colonies (*Lac*−

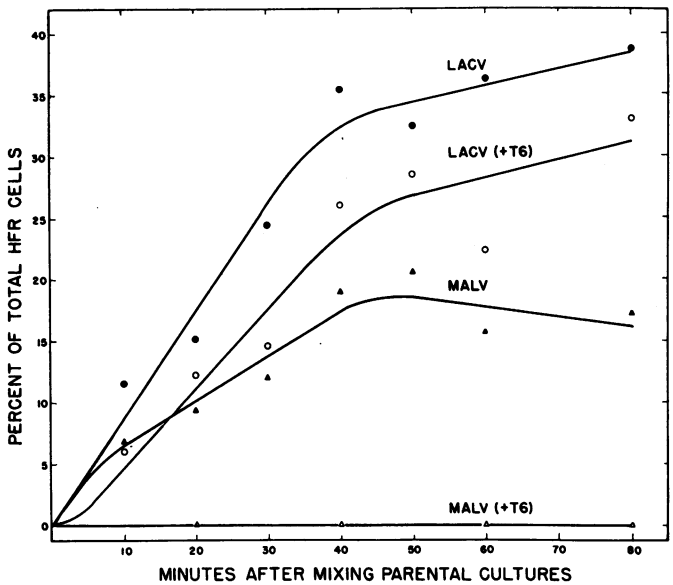


FIG. 1.—CS101 (*Hfr Lac*+ *Mal*+ $V_6^s$) × CS100 (*F*− *Lac*− *Mal*− $V_6^r$). The frequencies of variegated colonies (*Lacv* and *Malv*) appearing on EMB-lactose or EMB-maltose when samples of the parental mixture were plated at different times. The curves designated as *Lacv* (+T6) and *Malv* (+T6) were obtained when mixtures were exposed to T6 before plating.

as well as *Lacv*) were examined for the presence of the *Hfr* genes, $V_1^r$ and *L*+. $V_1^r$ was detected on EMB-lactose spread with about $10^9$ T1; *L*+ was detected on minimal medium (EMS-lactose[10]) supplemented with methionine, threonine, and thiamine (at concentrations of 10, 20, and 0.01 mg/l, respectively). The replica plates were scored after 18–24 hours' incubation at 37° C.

Colonies containing all seven possible combinations of the three *Hfr* genes scored (*Lac*+, $V_1^r$, and *L*+) were observed. Where two or three were present in a single clone, they were not invariably carried by the same subclone. Among those recombinant clones containing at least one of the three *Hfr* genes, three types predominated (94–99 per cent): Those which contained *Lac*+ but not $V_1^r$ or *L*+, those which contained *Lac*+ and $V_1^r$ but not *L*+, and those containing all three. As indicated in Figure 2 (*solid symbols*), the relative frequencies of these three

main types vary with the duration of mating prior to exposure to T6 and plating. This confirms the general conclusion of Wollman and Jacob that not all *Hfr* genes are transferred simultaneously. In addition, it provides evidence that transfer proceeds in a preferred sequence: *Lac* first, then $V_1$, and finally *L*.

The fact that this sequence of transfer is exactly opposite to the sequence suggested by the selected recombinants of Wollman and Jacob[5] requires explanation. The two sets of experiments differ in four important respects: the method of interrupting mating, the method of selecting recombinants, the *Hfr* strains used, and the lysogenicity of the strains used (Wollman and Jacob used nonlysogenic strains; all strains employed here are lysogenic for the temperate phage *lambda*). To determine the source of discrepancy in results, experiments were performed which used the T6-screening method described here and the lysogenic progenitor (W2323) of the *Hfr* strain employed by Wollman and Jacob. The results summarized in Table 1, although not extensive, appear to verify the temporal trend in gene frequencies reported by Wollman and Jacob. In particular, the fact that colonies containing *Lac*+ alone are always in a small minority, in contrast to the results obtained with CS101 (Fig. 2), indicates that differences between the *Hfr* strains (other than lysogenicity) are sufficient to explain the different sequences of transfer which have been observed. These differences may consist of structural inversions of the $Lac_1$—*L* region with respect to some point which is involved in the initiation of transfer. Jacob and Wollman[14] have discussed the possible significance of such structural differences in determining frequency of recombination.

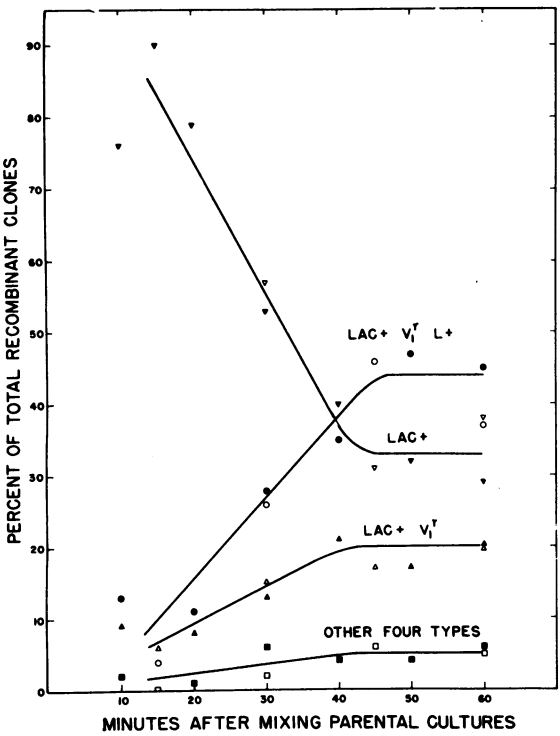


FIG. 2.—CS101 × CS100. The frequencies of different types of *F*− exconjugant clones obtained when samples of the parental mixture were plated at different times on nonselective medium. Each clone was scored for the presence of three *Hfr* genes: *Lac*+, $V_1^r$, and *L*+. "Total recombinant clones" is taken as the number which contained at least one of these genes. Solid symbols refer to clones obtained after T6 screening; open symbols refer to spontaneous exconjugant clones. At 10, 15, and 50 minutes, 50–100 clones were examined, at other times 100–300.

A reasonable model for the mating process has been afforded by observations on *Het* strain crosses (which yield persistent aneuploids).[11, 15] On this model, a complete *Hfr* (or *F*+) chromosome is transferred to a fertilized *F*− cell. Breakage then occurs at one or more *specific* points on this transferred chromosome. Reduc-

tion division follows rapidly, so that *Hfr* genes are lost unless crossovers have incorporated them into the complete *F*− chromosome. This explains the general predominance of *F*− markers in K12 crosses. The generality of this model has been questioned for some time,[16] but the experiments of Wollman and Jacob and ourselves provide the first strongly contrary evidence. This evidence consists of the fact that, to explain the temporal changes in gene frequencies, *varying* points of breakage of the *Hfr* chromosome must be assumed. If that part of the model based on *Het* crosses which calls for specific breakage points is not general, the generality of the part which calls for complete transfer of the *Hfr* genome is also open to question. The simpler alternative view that gene transfer may be normally partial becomes an economical working hypothesis.

TABLE 1

W2323 (*Hfr Lac* + *L* + $V_6^s$) × CS100 (*F* − *Lac*− *L*− $V_6^r$). Analysis of F-exconjugant clones containing either *Lac*+ or *L*+, obtained by exposure of the parental mixture to T6 after varying intervals of growth.

| Clones Which Contained | Duration of Mixed Growth (Minutes) 15 | 30 | 45 | 60 |
|---|---|---|---|---|
| *Lac*+ only | 0 | 5 | 3 | 4 |
| *Lac*+ and *L*+ | 5 | 31 | 43 | 58 |
| *L*+ only | 14 | 32 | 75 | 106 |
| Total clones examined | 19 | 68 | 121 | 168 |

However, in both the present experiments and those of Wollman and Jacob, exconjugants were obtained by artificial methods (exposure to phage or shearing in a blendor) which might induce abnormally partial transfer.[6] Therefore, an analysis was made of those exconjugants which arise spontaneously in a mating population of CS101 and CS100. This was done by plating samples at intervals directly onto EMB-maltose, where *F*− exconjugants and unmated *F*− cells form *Mal*− colonies. All colonies were then replica-plated to EMB-lactose, EMB-lactose plus T1, and EMS-lactose supplemented with methionine, threonine, and thiamine. Those *Mal*− colonies were then examined which contained one or more of the three *Hfr* genes, *Lac*+, $V_1^r$, and *L*+ (i.e., demonstrable exconjugants). The relative frequencies of combinations of *Hfr* genes among these exconjugant clones, plotted as open symbols in Figure 2, show that the same trend occurs whether or not mating is deliberately interrupted with phage. Therefore, discontinuities arise at varying sites in the *Hfr* genome under conditions corresponding closely to those prevailing in any bacterial cross, suggesting the normal transfer of partial genomes. In fact, the close agreement of trends among spontaneous and T6-induced exconjugants (Fig. 2) suggests that phage (and perhaps blending as well) may play no role in the *induction* of partial transfer but instead may screen for spontaneous exconjugants by rendering conjugating pairs incapable of producing recombinants.

*Summary.*—Nonsimultaneous gene transfer from *Hfr* to *F*− conjugating cells in *E. coli* K12 is confirmed. This transfer is oriented; certain genes regularly are transferred earlier than others. The sequence of transfer depends upon the *Hfr* strain employed, possibly due to structural differences. Sequential transfer is demonstrable among spontaneous exconjugants, strengthening the view that, normally, incomplete genomes are transferred.

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# *A PROPERTY OF SPECIAL JORDAN ALGEBRAS*

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Let $\mathfrak{M}$ be the set of all linear transformations on a finite-dimensional vector space over a field $\mathfrak{F}$ of characteristic not two. Then $\mathfrak{M}$ is an associative algebra with respect to the operation $ab$ of the ordinary product of linear transformations $a$ and $b$. But $\mathfrak{M}$ also forms a Jordan algebra $\mathfrak{M}^{(+)}$ with respect to the operation $a \cdot b = {}^1/_2(ab + ba)$.

At a recent Conference on Linear Algebras sponsored by the National Academy of Sciences–National Research Council, Nathan Jacobson conjectured that *every Jordan algebra $\mathfrak{B}$ is the homomorphic image of a special Jordan algebra $\mathfrak{A}$*, that is, the image of a subalgebra of a Jordan algebra $\mathfrak{M}^{(+)}$. In the present note we shall *disprove* the conjecture by showing that the known simple exceptional Jordan algebra is not such an image. Indeed, we shall derive the following more general result.

THEOREM. *Let $\mathfrak{B}$ be a Jordan algebra with radical $\mathfrak{H}$ such that $\mathfrak{B}$ is the homomorphic image of a special Jordan algebra $\mathfrak{A}$. Then $\mathfrak{B} - \mathfrak{H}$ is a special Jordan algebra.*

For $\mathfrak{A}$ is a Jordan subalgebra of an $\mathfrak{M}^{(+)}$ and has an enveloping associative subalgebra $\mathfrak{A}^*$ of $\mathfrak{M}$. Let $\mathfrak{N}$ be the radical of $\mathfrak{A}^*$, and form the semisimple associative image algebra $(\mathfrak{A}^*)' = \mathfrak{A}^* - \mathfrak{N}$. The elements of $(\mathfrak{A}^*)'$ are the cosets $x' = x + \mathfrak{N}$