CONTINUOUS CHROMOSOME TRANSFER IN *ESCHERICHIA COLI*

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THE current genetic picture of the process of sexual conjugation in *Escherichia coli* K-12, reviewed eloquently by JACOB and WOLLMAN (1961), is consistent with much evidence. The presence and position of a fertility episome, *F*, determines the transfer of the single linkage group from donor to recipient. Fertile F^+ cultures give rise to highly fertile Hfr mutants and to nonfertile F^- mutants. An Hfr mutant acts as a genetic donor, and in conjugation transfers its linkage group into a recipient, usually an F^- cell. The genetic transfer is polarized; an Hfr genome has an origin, O, the point on the linkage group at which transfer is initiated, and transfers successive gene loci ABC . . . Z with time until either transfer is interrupted spontaneously or artificially or the terminus of the linkage group is reached. The fertility episome, *F*, is linked at or near the terminus (Figure 1). Because spontaneous interruption occurs throughout the transfer process, most recombinants arise from partial zygotes. Most studies of genetic transfer have been concerned with transfer of the first 25% or less of the Hfr linkage group.

Different Hfr mutants were found to inject the linkage group in different arrangements, such as OABC . . . Z*F* and OBAZ . . . C*F*, and when several Hfrs were examined it became clear that the F^+ parent must have a closed, continuous linkage group from which the oriented, linear Hfr genome arises by the introduction of O . . . *F* (JACOB and WOLLMAN 1957; TAYLOR and ADELBERG 1960). The continuous linkage group is designated a circular chromosome. In an Hfr donor, the chromosome is open or opens for transfer from origin to terminus, and is injected progressively into the recipient, where it undergoes recombination with the recipient's chromosome.

WOLLMAN and JACOB (1958) found that when recombinants inherited a terminal marker such as Z of Figure 1, that marker was linked to markers near the origin, as A. "It seems accordingly that pairing between the Hfr and F^- chromosomes reestablishes the linkage relationships between the Z^+ and the A^+ characters which had been disrupted by the $F^+ \rightarrow$ Hfr mutation. This observation lends support to the hypothesis that, like an F^+ chromosome, the F^- chromosome is a closed, continuous structure" (JACOB and WOLLMAN 1961, p. 186). TAYLOR and ADELBERG (1961) used this interpretation to determine whether an Hfr had a closed or open chromosome when acting as a phenocopy recipient; they also observed linkage between A and Z. The interpretation given to the restoration of donor AZ linkage requires new assumptions. For Z to be linked to A, by definition the two markers must be integrated into the recombinant in

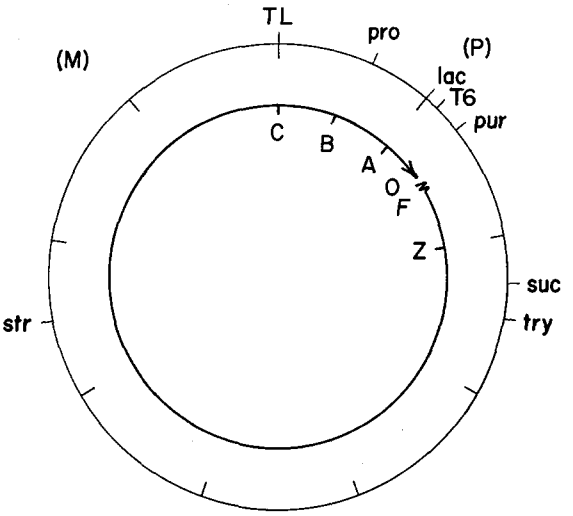

FIGURE 1.—Linkage map of *E. coli* K-12. The outer circle is a time of entry map for loci used in the initial crosses. In this and subsequent maps the circle is divided into nine 10-minute segments, giving a total map distance of 90 minutes (see text). Relative map distances are based on TAYLOR and THOMAN's (1964) synthesis, modified to include data obtained by me and by PITTARD, LOUTIT, and ADELBERG (1963). *P* and *M*, used for a special purpose, have not been precisely mapped in relation to the other loci.—The inner circle is a diagrammatic representation of the chromosome of HfrC, based on current hypotheses. O, origin; A, B, and C, markers near the origin; Z, a marker near the terminus; and *F*, the attached sex factor.

a dependent way. Marker A must not recombine with the F^- genome until Z is transferred 90 to 120 minutes later. In addition, either the donor A and Z must reunite (the circle must re-form) before recombination occurs, or A and Z regularly must both synapse with the continuous recipient genome in such a fashion as to restore linkage before recombination occurs. Of these two alternatives only the latter, implied by JACOB and WOLLMAN, requires a continuous recipient genome. In view of the uncertain nature of these and other possible assumptions, the observed restoration of donor AZ linkage is unexpected and basically unexplained.

In a study of the origin and terminus of one Hfr under conditions of complete chromosome transfer, I observed a restoration of AZ linkage that could best be explained by the hypothesis that chromosome transfer is continuous. A donor injects its chromosome to *F* and then initiates a second round of transfer, apparently without interruption, OABC . . . Z*F*ABC . . ., such that an Hfr has an origin but no terminus. The continuous transfer is interpreted in the context of JACOB and BRENNER's (1963) molecular model of genetic transfer.

Terminology: Most terms with meanings special to the K-12 mating system are used as defined by JACOB and WOLLMAN (1961). Because of the occurrence of unidirectional transfer, markers are distinguished by whether they enter the recipient earlier or later than a given reference marker. Proximal and distal have been frequently used to describe the relative position of markers (e.g., JACOB and

WOLLMAN 1961, p. 72), but they have to be uniquely defined in this system and become confusing when different donors are employed. The nautical terms fore and aft are convenient and precise. A fore marker enters the recipient before, and an aft marker after, a given reference marker. The same terms are used when the sequence of transfer is deduced from the pattern of transmission of markers to recombinant progeny.

Gene symbols are as defined by TAYLOR and THOMAN (1964), except in a few cases: *TL*, requirement for threonine and leucine, two genes here treated as a single marker; *M*, requirement for methionine; *P*, structural gene for alkaline phosphatase; and *T6*, resistance to phage T6.

The locus of the fertility episome in an Hfr donor is designated *hfr*. Two different donors were used, one of the CAVALLI type, HfrC, and one of the HAYES type, HfrH.

MATERIALS AND METHODS

Bacterial strains: The donors were CS101, the Hfr isolated by CAVALLI (see SKAAR and GAREN 1956). and AB301, an Hfr of the HAYES type independently isolated by DR. A. L. TAYLOR. Both are *M⁻ str-s*. A *T6-r* mutant and a *try⁻* mutant were isolated from CS101.

The recipient KB18 was prepared from the F⁻ 20GO of DRS. MONOD and JACOB (see BOICE and LURIA 1963). The markers *pro* and *M* were added by mating with a CS101 derivative, W4047R (BOICE and LURIA 1963), and the markers *pur*, *try*, and *suc* by mutation. The resulting strain was lysogenized with λ to yield KB18: *F⁻ pur⁻ T6-s lacZ*$_G^-$ *pro⁻ TL⁻ thi⁻ M⁻ str-r try⁻ suc⁻* (λ)+.

The recipient KB27 was prepared from AB1450 (PITTARD, LOUTIT, and ADELBERG 1963) by adding markers in the *pur-TL* segment from KB18 (via an HfrC recombinant of KB18) and lysogenizing with λ. KB27 has the genotype: F⁻ *pur⁻ T6-s lacZ*$_G^-$ *pro⁻ TL⁻ thi⁻ arg⁻ M⁻ ilv⁻ mtl⁻ xyl⁻ str-r his⁻* (λ)+ *gal⁻*.

All strains were *P*+, allelic *M⁻*, and (λ)+. Mutants were obtained by ethylmethane sulfonate treatment (STRAUSS 1962) followed by penicillin selection.

Media: The final concentrations of all media are given in grams per liter of distilled water.

Saline: NaCl, 8.5.

Penassay broth (Difco Antibiotic Medium 3): 17.5.

HA agar: Difco Antibiotic Medium 3, 17.5; Difco Bacto-Agar, 7.5.

BA agar: Difco Bacto-Tryptone, 10.0; NaCl, 5.0; Bacto-Agar, 11.0.

Minimal agar: KH_2PO_4, 3.0; K_2HPO_4, 7.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1 (after Davis and Mingioli 1950); glucose, 2.0; Bacto-Agar, 15.0. The agar (containing all added supplements), the salts, the glucose, and the $MgSO_4$ were combined after autoclaving separately. The supplements, added as necessary, were (in mg per liter): streptomycin, 200; adenine, 20; thiamine·HCl, 0.5; L-arginine·HCl, 100; L-histidine·HCl·H_2O, 40; L-isoleucine, 50; L-leucine, 40; L-methionine, 20; L-proline, 20; L-threonine, 80; L-tryptophan, 20; and L-valine, 50.

Sugar minimal: Na_2 succinate, 2.0, or galactose, 4.0, replaced the glucose in minimal agar.

MP (after GAREN and LEVINTHAL 1960): Difco Noble agar, 12.0, was autoclaved with nutritional supplements. A salt mix containing Sigma Trizma base (Tris hydroxymethyl aminomethane), 14.5; NaCl, 4.63; KCl, 1.50; $(NH_4)_2SO_4$, 2.63; in 1.0 N HCl, 87.5 ml, was autoclaved separately and added to the agar and supplements. Glucose, 2.0, and $MgSO_4 \cdot 7H_2O$, 0.1, were added from a sterile solution, and DL-β-glycerophosphate·2Na·$5H_2O$ (Sigma Chemical Co., Grade 1) was added from a filter-sterilized solution to a final concentration of 1.4×10^{-4} M.

EMB-sugar: Difco EMB agar base, 27.5; Difco yeast extract, 1.0; NaCl, 5.0. Sugar, 10.0, was added after autoclaving.

Mating procedure: Donor and recipient are mated, chromosome transfer interrupted after a specified time, and specific recombinants selected by their ability to grow where neither parent

can grow. Mating on the surface of membrane filters stabilizes conjugal pairs and reduces spontaneous interruption of chromosome transfer (MATNEY and ACHENBACH 1962). Overnight Penassay broth cultures of freshly recloned strains were diluted into Penassay broth (donor 1/50 and recipient 1/10) and incubated at 37°C with shaking for 2 hours. The growing cells were mixed by dilution into Penassay broth, and aliquots aspirated onto 25 cm HA Millipore filters to give an initial concentration of 2–3 $\times$ 10^7 donor cells and 4.5–6 $\times$ 10^8 recipient cells per filter. Each filter was immediately placed on an HA agar plate at 37°C and incubated at 37°C for the duration of mating. Zero minutes for each filter was the moment it was placed on agar; successive filters could be prepared in less than a minute each. After a given time of conjugation, a filter was removed from the agar, dropped into a 29 $\times$ 200 mm tube containing 10 ml of chilled saline, and agitated violently on a Vortex Jr. mixer for 30 seconds to suspend cells and separate pairs. With the donors used, these conditions of agitation were sufficient to interrupt mating. In crosses C and D, as an added precaution, 3 ml of the saline suspension was blended 30 seconds in a Lourdes multimixer with 15-ATT rotary knives at a transformer setting of 75. The suspensions were further diluted in saline and spread on the selective media of the experiment. Recombinant colonies were counted after 48 hours incubation at 37°C.

The frequency of recombination is the number of recombinants per 100 viable donor cells present at the start of mating.

Determination of recombinant genotypes: All recombinants include a segment of the donor chromosome (selected marker) and a segment of the recipient chromosome (counterselected marker). Recombinant genotypes reveal whether the remaining marked segments of their chromosomes come from donor or recipient.

Purification of recombinants was unnecessary in crosses A, B, and D. The donor parent was killed with streptomycin, and all unselected markers were arranged so that a recombinant with donor alleles could be determined even in the presence of recipient cells. A few percent of the recombinants had mixed genotypes. In cross C, recombinants were purified by streaking once on the selective medium. Crosses B (unpurified) and C (purified) gave very similar results, as is shown later (Table 5), eliminating any explanation which depends on recombinant colonies containing varying mixtures of genotypes. To avoid biased sampling, colonies were always picked from plates which had less than 150 recombinants, and all isolated colonies were picked until the desired number was achieved.

Isolated recombinant colonies were touched with a sterile toothpick and stabbed to a master plate of the same selective medium, 50 per plate. The master plates were incubated overnight at 37°C, and then replicated to test media with velveteen stamps (LEDERBERG and LEDERBERG 1952). The test media were: for auxotrophy, minimal medium deficient for the test nutrient; for succinate utilization, minimal medium with all supplements and succinate as the carbon source; for sugar fermentation, EMB with the test sugar; for resistance to phage T6, BA medium prespread with about 5 $\times$ 10^9 T6; and for donor, as described below. Replication to test T6 resistance or donor were always done last, and if both were done, with separate velvets. Replicates were incubated overnight and genotypes scored. Unless otherwise indicated, all genotypes could be scored without ambiguity.

Donor test: Those recombinants which receive the donor *hfr* character themselves become Hfr's (JACOB and WOLLMAN 1961, p. 183). Because all donor and recipient strains used in these experiments were $P+$ and allelic $M-$, all recombinants, independently of the rest of their genotype, were M^-P+. When mated to an $F^-M^+P^-$, only recombinants which have inherited donor *hfr* can produce $M+P+$ recombinants. The *P* gene is especially suitable, since it is linked to *lac* and in the region most frequently transferred by HfrC. $P+$ recombinants can be selected by growth on glycerophosphate as the sole source of phosphorus (GAREN 1960). The tester F^-, kindly provided by DR. ALAN GAREN, contains the nonreverting P^- allele E15 (GAREN and GAREN 1963), and has the genotype $M+$ *thi*$^-$ *TL*$^-$ P^- *str-r*. To test for the ability of recombinants to act as donors, overnight Penassay broth cultures of the tester F^- were washed to saline, and about 1 $\times$ 10^8 cells spread on an MP plate. The master plate was then replicated to this plate. Donors gave spots of confluent growth, while F^- recombinants gave only light residual growth. Replication to MP media without the tester F^- always gave no growth with recombinants from

the parent strains used, indicating that the M^- genes were indeed allelic and stable.

With HfrC, scoring of donors by this test was unambiguous. In several experiments recombinants from HfrC crosses which scored as donors were purified and further tested; all were found to have a gradient of transmission similar to HfrC.

RESULTS

In experiments initiated to examine the origin of HfrC, recombinants selected for donor markers TL^+ or pro^+ were found to inherit infrequently the donor allele of *pur*. The locus of *pur* was confirmed by recombination analysis and entry times to have the position given in Figure 1; *pur* is the most fore marker known in HfrC. With another Hfr each donor fore marker is inherited by at least half of the recombinants selected for an aft marker (JACOB and WOLLMAN 1961, p. 72); this was found true for all fore markers with HfrC except *pur*. The asymmetric inheritance of *pur* could be explained either by failure of some donors to transfer *pur*, or by universal transfer followed by exclusion from recombinants. The prezygotic interpretation suggests that the origin of HfrC might be variable, in that *pur* would sometimes be the first marker to enter, whereas at other times *T6* or *lac* would be. In cases where *T6* was the first entering marker, *pur* would be left at the terminus and might be transferred after *hfr* (see Figure 1). The F^- KB18 was synthesized to examine this possibility.

The accepted notion of a fixed origin leads to the conclusion that *pur* should enter recipients as a fore marker, linked to *lac*; if the origin is variable *pur* might also enter as an aft marker, linked to *hfr*. The relative entry position and linkage relationships were examined in cross A,

HfrC	*pur*+	*lac*+	*pro*+	*TL*+	*str-s*	*try*+	*suc*+	*hfr*+
F-	*pur*−	*lac*−	*pro*−	*TL*−	*str-r*	*try*−	*suc*−	*hfr*−,

by selecting pur^+, pro^+ and try^+ recombinants, with *str-r* counterselection, after prolonged mating on a Millipore filter. Since the time required for complete chromosome transfer has been estimated at about 110 minutes (cf. JACOB and WOLLMAN 1961), mating for 165 minutes was considered ample time to allow complete transfer. It was expected that pro^+ recombinants would show linkage to the *TL-pur* segment, but not to the *hfr-try* segment. If *pur* enters only early, pur^+ should show linkage only to the *TL-pur* segment, and try^+ only to the *hfr-try* segment, but if *pur* can also enter as an aft marker, try^+ and pur^+ recombinants should show linkage in the *pur-try* segment. This is based on the assumption that the origin and terminus of an Hfr interrupts linkage. If this is the case, no recombinants should show linkage throughout the *TL-try* segment. The *TL-pur* segment was marked to determine the point of interruption of linkage: between *pur* and *hfr*, or if the origin is variable, between *lac* and *pur*. These predictions do not consider the previous descriptions of AZ linkage, about which limited data has been published, and the influence of AZ linkage on the expected results had to be considered simultaneously. The results of cross A (Table 1) confounded any simple interpretation.

The asymmetrical inheritance of *pur* can be seen in this experiment. Even

TABLE 1

Cross A: Effect of selected marker on genotypic constitution

Donor marker selected	Percent recombination	Genotypic constitution (% donor marker)								
		pur	*lac*	*pro*	*TL*	*try*		*suc*		*hfr*
pur	17	100	35	24	9	4		4		8
pro	50	7	77	100	30	1	=	1	=	1
try	0.8	55	52	50	37	100		98		55*

* 54 of 55 *hfr*$^+$ *pur*$^+$.

CS101 and KB18 were mated on a Millipore filter for 165 minutes, then plated on media to select recombinants receiving the indicated donor marker. Donors were counterselected with streptomycin. One hundred recombinants of each type were tested.

though *pur* enters fore of *pro*, *pro*$^+$ recombinants are three times as frequent as *pur*$^+$. Among *pro*$^+$ recombinants, donor *lac* is frequent but only 7% inherited donor *pur*. These two asymmetries are unique to *pur*.

The pattern of the *pro*$^+$ recombinants is as anticipated. One recombinant received the donor *try-hfr* segment; this is reasonable on the basis of the relative frequency of the *try*$^+$ recombinants (1.6% of *pro*$^+$). The *pur*$^+$ recombinants are typical as far aft as *TL*, but 4% inherited the *try-hfr* segment, and 4% more just *hfr*. The increase in *hfr*$^+$ above those obtained with *pro* selection, while barely significant, supports the notion that the increase results from *pur* which enter aft of *hfr*.

Among the *try*$^+$ recombinants, 54% were *hfr*$^+$ *pur*$^+$ indicating a strong *hfr-pur* linkage. Though some linkage was a possibility, this is much more than expected, even if, for example, *pur* enters aft 80% of the time. But the linkage was not discontinuous: *pur* was linked to *lac*, *lac* to *pro*, etc., so that the linkage relationship became *try suc hfr pur lac pro TL*. This marked continuity of linkage across the origin and terminus confounded any attempt to test the variable origin hypothesis by this approach. Analysis of the genotypes of the *try*$^+$ recombinants suggested two alternatives: (1) When complete transfer occurs, the donor genome restores AZ linkage, resulting in continuous linkage across the *try-TL* segment. This would constitute a confirmation of the previously observed AZ linkage, and would require the same assumptions to interpret it. (2) When one round of transfer is complete, another round begins at once, so that transfer is continuous, with an Hfr having an origin but no terminus. Linkage in the *try-TL* segment would result from a second round of entry of *pur-TL* (Figure 2).

The two genetic models could be distinguished readily. Recombinants selected for a given donor marker will display, with increasing time allowed for transfer, an increasing frequency of those markers which enter aft of the selected marker. The first model leads to the prediction that when *hfr* enters transfer is complete or nearly complete, so linkage relationships should not continue to change with time. In contrast, the second model suggests that after *hfr* enters a second round commences, and subsequent aft markers should be introduced in an orderly sequence with time.

If, with the markers used in cross A, the *pur-TL* segment enters only fore of

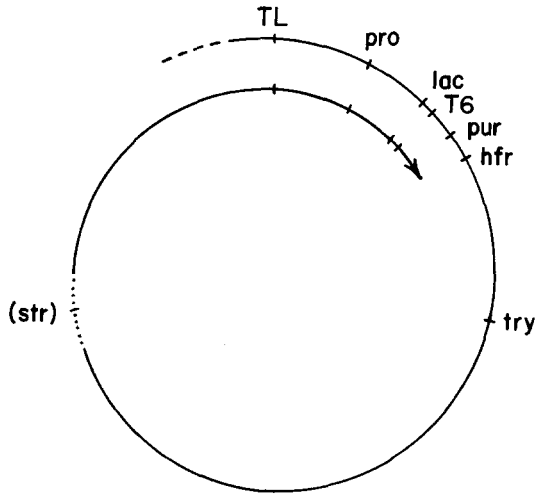

FIGURE 2.—Continuous chromosome transfer, showing the postulated pattern of transfer of the donor linkage group with an origin (arrow) but no terminus. The markers are those used in crosses A and B, placed in proportion to their relative distances with one complete linkage group of 90 minutes as in Figure 1. In cross B, *try* is the selected marker, and *str* the counter-selected marker. For this reason, the *str* region of a recombinant must come from the recipient genome.

try (model 1), the inheritance of these markers linked to *hfr* should remain at a constant value, whereas if they enter aft of *hfr* as well (model 2), their inheritance among recombinants with donor *hfr* should continue to increase with time. To distinguish these possibilities, cross B was performed with the same strains as cross A, with the addition that the donor was also *T6-r* and the recipient *T6-s*. Mating was interrupted at 90, 105, 120, and 150 minutes, and try^+ *str-r* recombinants selected. Since complete transfer was expected to take about 110 minutes, no recombinants were expected at 90 minutes, a few try^+ at 105 minutes, and increasing numbers of try^+ hfr^+ at 120 and 150 minutes. The critical question was what happened to the inheritance of the *pur-TL* segment among these try^+ hfr^+.

In cross B, try^+ recombinants were already present in very small numbers by 90 minutes, and increased in number throughout the time allowed for mating (Figure 3A). There is a tremendous heterogeneity in the time of entry of *try* to different zygotes; the time of entry curve for a fore marker such as *pro* would be almost vertical on the time scale of Figure 3A. Extrapolation of the steepest slope to the origin gives a time of entry for *try* of 105 minutes, as expected, but it is clear that some zygotes receive *try* as early as 90 minutes. Control platings of the two parents indicated that none of the try^+ *str-r* were due to mutation. Unfortunately, few recombinants were obtained when transfer was interrupted at 90 or at 105 minutes, so these two samples were pooled for the subsequent analysis.

The genotypic constitution of the recombinants changed markedly with increased mating time (Table 2). Almost all try^+ were suc^+, which confirms that

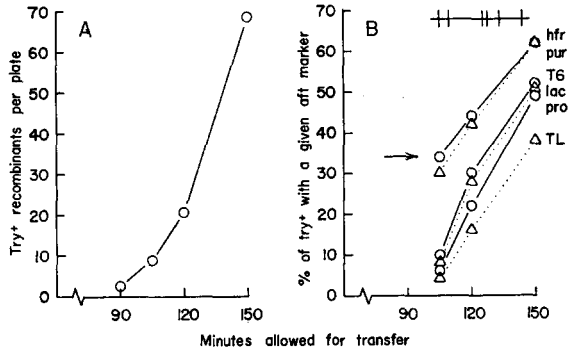

FIGURE 3.—Time of entry curves for cross B. Curve A shows the appearance of *try*+ *str-r* recombinants in number per plate at a constant dilution such that 1.6×10^4 of the viable donor cells present initially were plated on each. At 150 minutes there were 0.43% recombinants. Curve B shows the appearance of given donor markers among the aft symmetry classes of Table 3. The 90 and 105 minute samples are pooled and plotted at 105 minutes. Above the graph is a "map" showing the relative distances, from left to right, of *hfr pur T6 lac pro TL*, as interpolated at the point indicated by the arrow.

all were *try*+ recombinants rather than revertants. The close linkage of *try* and *suc* under these conditions renders *suc* useless as a locus marker.

The only useful aft marker is *hfr*. As expected for an aft marker, the proportion of recombinants which were *hfr*+ increased with time. The presence of some *hfr*+ in the 90 minute sample (6 of 12 recombinants) suggests that a few donors transferred the complete genome within 90 minutes.

The proportion of each of the donor fore markers among the recombinants also increased with time as did the aft marker *hfr*, but with a gradient: *pur* being most like *hfr* and *TL* least similar. Without reference to other map data, a probable order of loci is *try suc hfr pur T6 lac pro TL*.

If continuous transfer is the correct explanation, markers in the *pur-TL* segment have the potential to enter the recipient twice when *try*+ recombinants are selected. These markers should always enter the recipient as fore markers, and they could also enter aft of *try*. Recombinants are the end product, and there is no way to tell, by examining recombinant genotypes, whether a normally fore marker entered the recipient fore (first round) or aft (second round) of *try*. It

TABLE 2

Cross B: Effect of time allowed for transfer on genotypic constitution

Minutes of transfer	Genotypic constitution (% donor marker)							
	pur	*T6*	*lac*	*pro*	*TL*	*try*	*suc*	*hfr*
90–105	32	26	28	22	16	100	100	34
120	48	42	40	36	26	100	98	46
150	68	61	62	64	51	100	100	67

CS101 *T6-r* and KB18 were mated on Millipore filters, then at the indicated times separated by agitation and plated to select *try*+ *str-r* recombinants. Fifty recombinants each were picked for analysis from the 90–105 minute (12 and 38 recombinants, respectively) and the 120 minute samples, and 100 from the 150 minute sample.

TABLE 3

Cross B: Recombinant genotypes arrayed by fore and aft symmetry

Symmetry	Donor markers inherited	Percent of recombinants 90–105 min	120 min	150 min
	pur T6 lac pro TL try	0	2	0
	T6 lac pro TL try	6	4	4
FORE	*lac pro TL try*	4	0	1
	pro TL try	0	0	0
	TL try	0	2	2
CENTRAL	*try*	44	36	19
	try hfr	4	2	0
	try hfr pur	20	12	10
	try hfr pur T6	2	2	1
AFT	*try hfr pur T6 lac*	2	6	2
	try hfr pur T6 lac pro	2	6	11
	try hfr pur T6 lac pro TL	4	16	38
	Other fore combinations	12	10	7
	Mixed combinations	0	2	5
		100	100	100

is possible, however, to arrange the recombinants according to the symmetry of their specific genotypes (Table 3). By selection all must inherit donor *try*, the central marker in the arrangement. *Hfr* is aft of *try*, and near or at the end of the complete linkage group (confirmed in this system), so any recombinant with donor *hfr* has inherited at least one marker aft of *try*. On the basis of *hfr*, the recombinants can be divided into fore and aft symmetry. Those which remain F^- (*hfr*$^-$) have no known aft marker (excluding *suc*, which is very close to *try*), and thus have fore symmetry, whereas those which become *hfr*$^+$ have an aft symmetry. Arrangement by these simple criteria gives the pattern of Table 3, which shows the complete genotypes for those recombinants which inherited continuous segments of donor genome. Such recombinants are in a majority (88%). Of five possible fore symmetries, four were found. In addition, "other fore combinations" include those recombinants with fore symmetry (*hfr*$^-$) which inherited a mixed portion of the *pur-TL* segment. These other fore combinations included 11 of 27 possible distinct genotypes among 9% of all recombinants. The most frequent (2.5%) inherited donor *T6 lac pro try*, but not *pur* or *TL*. All possible aft symmetries were found. In addition, 3% of all recombinants were *hfr*$^+$ but contained "mixed combinations" of fore and aft symmetry. The mixed combinations included four genotypes:

T6 lac pro TL ... try hfr
lac pro TL ... try hfr
pro TL ... try hfr
pro TL ... try hfr pur T6.

In computing proportions, the mixed combinations are included in both fore and aft symmetries.

These patterns are readily understood if the symmetry of recombinant genotypes reflects the order of genetic transfer. Markers transferred fore of *try* should be inherited by a constant proportion of the zygotes when *try* is the selected marker. Since the use of *str* counterselection forces a switch to the recipient *str* region (see Figure 2), the expected constant proportion of fore markers should be low. Recombinants with fore symmetry accounted for 22, 20, and 19% of the recombinants at the three successive times of interruption.

In contrast, recombinants with central and aft symmetry should shift with time, as transfer progresses from *try* to *hfr* and on to *TL*. Such a shift was observed, with the central class decreasing and the aft classes progressively increasing with time (Table 3). The hypothesis that recombinants which receive the "terminus" somehow reestablish AZ linkage, and thus inherit an increased proportion of donor fore markers, is insufficient to explain these progressive shifts among aft symmetry recombinants. If it were sufficient, the proportion of try^+ hfr^+ recombinants inheriting portions of the donor *pur-TL* segment should remain constant with time.

Recombinants with aft symmetry are remarkable in that they inherit, *en bloc*, complete segments of the donor linkage group. Of the 200 recombinants studied, 101 were aft and six were mixed. All of the aft symmetry recombinants, by definition, inherited a complete, unbroken donor segment of length determined by the two outside markers. The six mixed combinations observed could represent either switches within aft symmetry, which would reduce the inheritance of complete segments to 101 of 107 or 95%, or true mixtures of fore and aft. The specific genotypes of mixed combinations are interpreted to favor the latter, which suggests that linkage from *try* to the terminal inherited marker is complete. This degree of linkage is in contrast to the diversity of genotypes among the recombinants with fore symmetry, and to the loose linkage usually observed in *E. coli* K-12 crosses.

The probability of spontaneous interruption of the continuity of transfer or integration is low in the aft classes. The majority of aft recombinants at 150 minutes inherited the whole *try-TL* segment. In addition, the *try hfr* and *try hfr pur* classes actually decline during the course of continued mating (Table 3). These declines are best explained if there is a high probability of continuation, so that these classes, involving closely linked markers (Figure 2), are basically only obtained when transfer is interrupted by blending. All aft classes show a changing pattern consistent with a high probability of continued transfer.

The small, constant proportion of fore symmetry classes, the decrease in central and especially the progressive increase in aft classes, all support the assumption that the array of recombinants in Table 3 does reflect the order of transfer. If so, it should be possible to construct a crude time of entry curve for the second round of entry. WOLLMAN, JACOB, and HAYES (1956) have shown that the percent of aft donor markers among recombinants selected for a fore marker increases with increased mating time before blending, permitting the construction of a time of entry map analogous to those obtained from direct blending. The percentage of each donor marker among the recombinants with aft symmetry

(Table 3) was summed and plotted. The resulting curves (Figure 3B) do not intersect the abscissa, so conventional mapping, which uses the point of initial rise (JACOB and WOLLMAN 1961, p. 133; TAYLOR and THOMAN 1964) cannot be used. All curves can be interpolated at the percentage indicated by the arrow (Figure 3B) to give the map displayed above the curves. Since this map reflects the great population heterogeneity, the time map is considerably spread when compared with a conventional map, the distance from *hfr* to *TL* being 40 minutes instead of the approximately 15 minutes obtained from conventional mapping. In spite of this, the relative map distances agree excellently (cf. Figure 2), except that the relative *pur-T6* distance is nearly three times its expected length. The curves display an unambiguous ordered sequence of markers, *try hfr pur T6 lac pro TL*, strongly supporting the hypothesis that chromosome transfer is continuous.

Two additional crosses, with another F$^-$, were performed to confirm and extend the results of cross B. The extent of transfer was examined in cross C (Figure 4A),

HfrC	*pur*+	*lac*+	*pro*+	*TL*+	*arg*+	*ilv*+	*xyl*+	*str-s*	*his*+	*try*$^-$	*gal*+	*hfr*+
F$^-$	*pur*$^-$	*lac*$^-$	*pro*$^-$	*TL*$^-$	*arg*$^-$	*ilv*$^-$	*xyl*$^-$	*str-r*	*his*$^-$	*try*+	*gal*$^-$	*hfr*$^-$,

by selecting *gal*+ *try*+ recombinants after mating was interrupted by vortexing and blending. Recombinants appeared at 90 minutes, and increased in number thereafter (Table 4). The rise after 150 minutes is believed to be the result of growth of recombinants (WOLLMAN, JACOB, and HAYES 1956; TOMIZAWA 1960), so that an increasing proportion of the colonies studied after 150 minutes represent recombinant progeny. When mating was interrupted at 60 minutes, there were no recombinants at the standard dilution, though there were 0.01% recombinants at a higher cell concentration per plate. These were not revertants, and they may represent very early transfer, the temporary or permanent equivalent

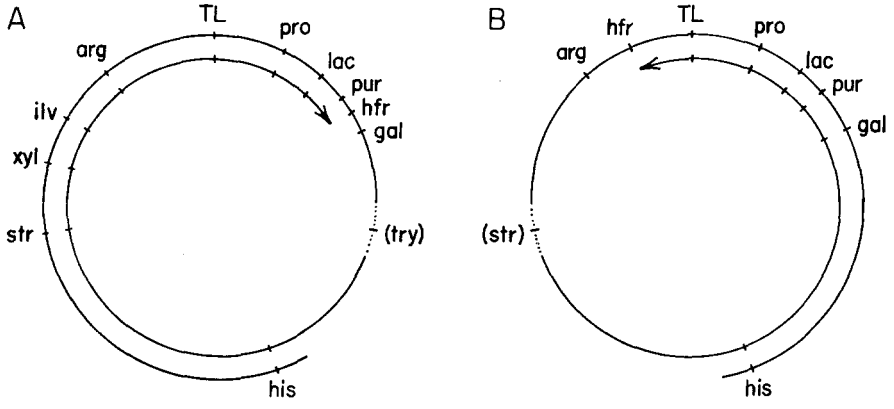

FIGURE 4.—Continuous chromosome transfer model showing the marked loci used in cross C (A) and in cross D (B). The relative lengths of the donor linkage group transferred from the origin (arrow) are required to explain the recombinant genotypes. Dotted segments show the counterselected markers, which must come from the recipient.

TABLE 4

Analysis of cross C

Symmetry	Donor markers inherited	Percent of recombinants				
		90 min *0.08%	120 min 0.34%	150 min 1.48%	180 min 1.87%	210 min 2.76%
FORE	all combinations (44 genotypes)	7	11	17	12	12
CENTRAL	*gal*	22	15	5	10	8
AFT†	*gal hfr*	14	6	3	3	9
	gal hfr pur	38	16	18	21	13
	gal hfr pur lac	6	3	5	5	3
	gal hfr pur lac pro	7	18	15	11	15
	gal hfr pur lac pro TL	4	10	11	8	16
	gal hfr pur lac pro TL arg	0	5	2	4	6
	gal hfr pur lac pro TL arg ilv	1	3	4	3	3
	gal hfr pur lac pro TL arg ilv xyl	0	5	4	8	3
	gal hfr pur lac pro TL arg ilv xyl str	1	2	7	4	5
	gal hfr pur lac pro TL arg ilv xyl str his	0	6	9	11	7
		100	100	100	100	100
Percent of aft combinations which were mixed (72 genotypes)		9	28	30	30	33

* Percent recombination.

† Aft symmetry classes include mixed combinations, i.e., those containing the complete sequence given plus one or more isolated donor markers. Most of the 72 mixed genotypes were observed only once among the 500 recombinants. The most common one, accounting for 2% of all recombinants, contained donor *gal hfr pur lac pro . . . his*. The percentage of mixed combinations is given at the bottom of the table.

CS101 *try*$^-$ and KB27 were mated on Millipore filters, and after intervals at 37±1°C individual filters were removed to saline, the cells suspended and mating pairs separated by vortexing and blending, and dilutions plated on medium to select *gal*$^+$ *try*$^+$ recombinants. After counting, 100 colonies from each time were purified, the reisolated colonies transferred to master plates and replicated to various test media.

of F′ transfer (JACOB and ADELBERG 1959), plate matings, or even unseparated pairs. Their frequency is too low to seriously influence the genotypes of the recombinants studied. From these results it may be concluded that separation of pairs was efficient, that revertants and plate matings were negligible, and therefore that the recombinant genotypes reflect the inheritance patterns of separated pairs.

Among the 500 recombinants with ten unselected markers, 127 of 1024 possible specific genotypes were observed—most of them only once. Genotypes representing pure central and aft symmetries were the most frequent. A brief tabulation is given in Table 4. All fore genotypes are pooled, since none of the 44 genotypes were common. The aft symmetry classes include mixed combinations, as defined in the footnote, and therefore bring in the interesting portion of 72 uncommon genotypes. No pattern comparable to Table 3 emerges even in this summary tabulation because the differences are spread through so many unselected markers.

Cross C confirms the results of cross B, but transfer was accelerated (Table 5). The comparison shows that cross C had reached about the same point by 120 minutes that cross B reached in 150 minutes. The relative closeness of the selected markers, *gal* vs. *try*, to *hfr*, or a slight difference in the temperature during mating (cf. HAYES 1957) would explain this difference. Most important, cross C

TABLE 5

Comparison of crosses B and C

Donor markers inherited	Percent of recombinants			
	90 min	120 min	150 min	180 min
sel hfr	14	6	3	3
	4	2	0	
sel hfr pur	38	16	18	21
	22	14	11	
sel hfr pur lac	6	3	5	5
	2	6	2	
sel hfr pur lac pro	7	18	15	11
	2	6	11	
sel hfr pur lac pro TL	6	31	37	38
	4	16	38	

Data for cross B are given directly beneath that for cross C. In both cases the results, from Tables 3 and 4, are summed so the aft symmetry classes would be those observed if no other unselected markers were included in the crosses. The selected marker, *sel*, was *try* in cross B and *gal* in cross C. The pooled 90 and 105 minute sample of cross B is given under 90 minutes.

showed the same pattern of shifts as cross B. The result also demonstrates that aft symmetries accumulated to *TL* in cross B simply because *TL* was the last marker in the sequence (cf. Tables 3 and 4).

The progressive shifts in cross C can best be seen in an abridged form (Table 6). By 90 minutes, transfer is complete to *hfr* and almost to *pur* (which is only 3 to 4 minutes from *gal*). By 120 minutes, transfer has progressed to *pro*, and almost to *TL*; and by 150 minutes, it is complete to *str* and almost, if not, complete to *his*. There are no major changes after 150 minutes. Because the early

TABLE 6

Cross C: Cumulative aft entry

Genotypes complete to or beyond donor marker	Map distance in minutes	Percent of recombinants		
		90 min	120 min	150 min
hfr		71	74	78
	2			
pur		57	68	75
	7			
pro		13	49	52
	6			
TL		6	31	37
	15			
ilv		2	16	24
	10			
str		1	8	16
	25			
his		0	6	9

The aft symmetry recombinants given in Table 4 were used to compute the number that received a given donor marker. Map distances between markers are from conventional (fore) time of entry experiments and are approximate. There were no marked changes after 150 minutes.

markers had progressed so far by 90 minutes, a useful time of entry map is not possible, but detailed analysis of the genotypes reveals a single consistent sequence *gal hfr pur lac pro TL arg ilv xyl str his.*

Aft symmetry classes accumulate with time from *gal* to *his*, and from 150 minutes on about 9% of the recombinants inherit the complete *gal-his* segment. This indicates that the second round of transfer does not stop at *TL*, and extends the distance to more than two thirds of the total map. It is impractical to attempt to extend the demonstration any further, since it would not be possible to go beyond the counterselected marker.

The results of cross C thus support cross B, and extend continuity for 10 unselected markers in sequence to span at least 66% of the linkage group. This supports the model of continuous transfer, and suggests that a donor has *no* fixed terminus. Linkage of markers in the aft symmetry classes is again marked (Table 4), though determination of whether it is absolute depends on the interpretation given to mixed combinations. The detailed analysis of cross C, from which this summary is drawn, does not alter these conclusions.

Cross D was performed to determine whether these results could be generalized to a different Hfr, transferring in a different direction (Figure 4B),

HfrH	*TL*+	*pro*+	*lac*+	*pur*+	*gal*+	*his*+	*str-s*	*arg*+	*hfr*+
F⁻	*TL*⁻	*pro*⁻	*lac*⁻	*pur*⁻	*gal*⁻	*his*⁻	*str-r*	*arg*⁻	*hfr*⁻,

with *arg*+ *str-r* recombinants selected. The results, summarized in Table 7, show that: (1) recombinants are present by 90 minutes; (2) aft classes show progressive shifts with time in an ordered sequence, from *arg* through *his*; and (3) there

TABLE 7

Analysis of cross D

Symmetry	Donor markers inherited	Percent of recombinants		
		90 min *0.06%	120 min 0.31%	150 min 1.14%
FORE	all combinations (19 genotypes)	4	22	10
CENTRAL	*arg*	34	16	8
AFT†	*arg hfr*	39	28	28
	arg hfr TL	16	10	9
	arg hfr TL pro	0	6	3
	arg hfr TL pro lac	1	4	7
	arg hfr TL pro lac pur	2	7	6
	arg hfr TL pro lac pur gal	4	5	14
	arg hfr TL pro lac pur gal his	0	2	15
		100	100	100
Percent of aft combinations which were mixed (24 genotypes)		5	20	21

* Percent recombination.
† See footnote to Table 4.
AB301 and KB27 were mated on Millipore filters, and after intervals at 37°C the cells suspended and mating pairs separated, and dilutions plated on medium selective for *arg*+ *str-r* recombinants. One hundred recombinants from each time were tested. The scoring of donors was much less satisfactory than in crosses with HfrC, for which the donor test was developed, so that about 10% of the scoring of *hfr* was uncertain. This is not sufficient to influence the patterns.

TABLE 8

Linkage across the origin

Cross	Donor	Selected marker	Percent *hfr*+	Percent of *hfr*+ with donor	
				first fore marker	*lac*
B	HfrC	*try*	67	96	78
C	HfrC	*gal*	78	96	73
D	HfrH	*arg*	82	66	51

The estimate is based on the proportion of *hfr*+ recombinants which have also received a given fore marker from the donor. Data for 100 recombinants each after 150 minutes of mating are from Tables 3, 4, and 7. For HfrC the first fore marker is *pur*; for HfrH, *TL*.

is marked *en bloc* inheritance among the aft classes. This extends the basic features of continuous transfer to HfrH.

Comparison of linkage across the origin as observed at one time point in crosses B, C, and D shows that in all crosses the linkage between *hfr* and the first fore marker was great (Table 8). The percentage of recombinants which became *hfr*+ appears related to the closeness of *hfr* to the selected marker in each cross. With HfrC, linkage of *hfr* and *pur* is very marked, which is expected since the two markers are about 2 minutes apart (Figures 2 and 4A). Since the original hypothesis of a variable origin suggested that *pur* could enter as an aft (and terminal) marker, the linkage of *hfr* to another fore marker, *lac*, which on any hypothesis seems always to enter as a fore marker, is also examined. With HfrH, where the distance from *hfr* to the first fore marker is greater, the linkage is less. In all cases, the observed linkage seems related to the distance, and in all cases the linkage of *hfr* to the first definite fore marker is greater than 50%.

DISCUSSION

The progressively shifting pattern of recombinant genotypes in these crosses indicates that after the completion of one round of transfer, transfer can continue with a second round. This suggests the corollary that the linkage group of a donor remains continuous during transfer, with an origin but no terminus (Figures 2 and 4). Continuity replaces the notion that the donor linkage group is a linear structure derived by opening the continuous linkage group at *F* (Figure 1).

The argument for continuous transfer, presented with the crosses, is based on the assumption that the change in recombinant genotypes with time reflects prezygotic rather than postzygotic events. That is, if the pattern of transmission reflects that of transfer, as it does in conventional crosses (JACOB and WOLLMAN 1961, p. 152), then the results demonstrate that chromosome transfer is continuous. Because recombinants are static, it remains possible to explain the results within the accepted notion that chromosome transfer proceeds to the terminus at *hfr*, never further. This requires several assumptions. (1) When *hfr* enters a zygote, linkage is restored between *hfr* and fore markers; this requires in turn the assumptions given in the introduction. (2) The reestablishment of linkage must be virtually obligatory to account for the observed frequency, always greater than

50% (Table 8). (3) Conditions must progressively change during prolonged mating on HA agar in a way which markedly affects the integration of donor markers into recombinants. In those zygotes which reestablish continuity, linkage of donor markers must be progressively tightened in a gradient progressing along the chromosome from *hfr*, such that the pattern of inheritance with time would change through ZF, ZFA, ZFAB, etc. Integration of donor *hfr* and increasing linkage would then determine the pattern, rather than the time of entry. It would be remarkable if all these assumptions were valid, and especially if a gradual environmental change were so poised as to result in a progressive integration of donor markers into recombinants, permitting in its extreme the construction of an aft time of entry map. While the postzygotic interpretation is conceivable, continuous transfer is a more economical hypothesis.

These two interpretations reflect the possible time of entry of normally fore markers with respect to *hfr*: entry only fore of *hfr*, as in the conventional notion, or both fore and aft, as in continuous transfer. A third alternative would be that the pattern results from entry of fore markers both fore and independently, as might occur in a donor with two origins, such as the double male described by CLARK (1963). This alternative is easily eliminated, since with the donors used in these experiments all markers examined are transferred and inherited as members of a single, consistent, continuous linkage group.

The tight linkage across the "origin" argues that the transfer occurs without a break in the continuity, as Figures 2 and 4 suggest, rather than by one round of transfer followed by a second.

Continuous transfer explains the previous observations of linkage across the origin (WOLLMAN and JACOB 1958; TAYLOR and ADELBERG 1961). In TAYLOR and ADELBERG's experiments, with a different Hfr than the two used here, as many as 70% of the recombinants selected for an aft marker inherited a fore marker, which fits excellently with the present results.

The experiments were initiated to determine whether the asymmetrical inheritance of *pur* with HfrC was due to a low probability of (a) transfer of *pur* into recipients, or (b) integration of *pur* into recombinants. The occurrence of continuity leaves this an open question.

A byproduct of this study is the observation that transfer of the complete chromosome can occur within 90 minutes. In the three interrupted matings, a high proportion of the recombinants selected after 90 minutes of contact inherited donor *hfr*, and some, according to the continuity hypothesis, have aft symmetries indicating further transfer within that time. The ability of infrequent donors to complete transfer in 90 minutes agrees with the shortened cumulative map distance of 87 minutes reported by TAYLOR and THOMAN (1964). The great heterogeneity in the time of entry of aft markers might support the idea that genetic transfer does not proceed at a constant rate (JACOB and WOLLMAN 1958; DE HAAN and GROSS 1962), but could result simply from changing transfer conditions due to growth of cells on the membrane filter during mating.

The *en bloc* integration of large segments of the donor chromosome into recombinants with aft symmetry is in marked contrast to the loose linkage usually observed in *E. coli* K-12 crosses and found in these crosses for pairs of markers

not involved in *en bloc* integration (whether in fore or aft mixed classes). The mechanism of integration may be different in the two cases, or the difference may be a result of the different sizes of the segments examined—a difference which is known to affect linkage relationships in *E. coli* (see JACOB and WOLLMAN 1961, p. 226; MACCACARO and HAYES 1961).

The presence of several homologous chromosomes per cell (e.g., WITKIN 1951) permits the elaboration of two models involving interaction of pairs of donor chromosomes to explain continuous transfer. These models are similar to those devised to interpret chromosome transfer in F′ strains (SCAIFE and GROSS 1963; PITTARD and ADELBERG 1964). In the first model, all chromosomes in a donor cell open upon conjugation to yield linear OA . . . *ZF* structures. Once one of the chromosomes begins to be transferred, all chromosomes have a tendency to close again to restore *ZF*A linkage. If, instead of closing with itself, a chromosome not being transferred closes with the one being transferred, the required OA . . . *ZF*A . . . *ZF* would be created. If closure with self or nonself are equally probable, these double chromosomes would occur in less than 50% of the donor cells. In the second model only that chromosome being transferred opens; the others remain closed (the second model only works with this exceptional assumption). The linear chromosome being transferred can recombine with a continuous chromosome remaining in the donor, and appropriate recombination yields OA . . . *ZF*A . . . *ZF*. Since only odd numbers of switches yield this form, it should occur in no more than half of the donors. The results of the crosses, if interpreted as continuous transfer, show that many more than half the zygotes receiving *hfr* initiate a second round, which argues against such models unless additional assumptions are made.

A physical basis for continuous chromosome transfer may be found in the recent hypothesis of JACOB and BRENNER (1963; JACOB, BRENNER, and CUZIN 1963) that chromosome transfer occurs by DNA replication. The chromosome is viewed as a single DNA duplex. JACOB and BRENNER have postulated that conjugation initiates replication of the Hfr chromosome beginning at the attached *F* factor and progressing aft from the origin. As replication of the linear Hfr chromosome proceeds, the process of replication injects one of the two daughter chromosomes into the F^-, while the other remains in the Hfr. This hypothesis is receiving considerable support (L. G. CARO and J. D. GROSS, 1964 personal communication; JACOB, BRENNER, and CUZIN 1963; PTASHNE 1965; ROESER and KONETZKA 1964). NAGATA (1963) found, however, that during the exponential growth of an Hfr, the linkage group replicates from the terminus toward the origin. This replication for growth, opposite in orientation to replication for transfer, could be reconciled if two different replication systems, both starting at *F*, are employed for growth and for genetic transfer.

When cells of an Hfr strain of *E. coli* K-12 are growing exponentially, the chromosome appears to remain as a continuous DNA duplex, and is replicated at a single growing point which moves along the chromosome (CAIRNS 1963a, b). The process of DNA replication occupies most of the division cycle (MAALØE 1961).

Combination of the hypothesis of transfer by replication with the hypothesis

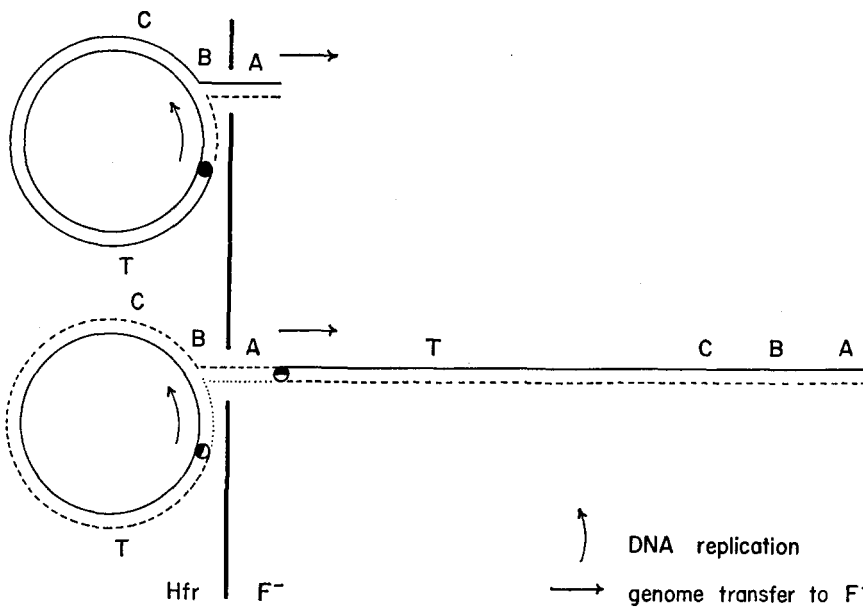

FIGURE 5.—Continuous chromosome transfer by DNA replication. One strand is imagined to open upon conjugation, and to be replicated into the recipient—after JACOB and BRENNER (1963). The remaining strand remains continuous—after CAIRNS (1963b). A, B, C, and T are markers. The upper diagram might represent the situation at 10 minutes, the lower at 100 minutes. For convenience the helix is unwound, and the attached *F* is distinguished as a small circle.

that an Hfr chromosome remains continuous even during transfer yields the model shown in Figure 5. Upon conjugation the donor DNA chromosome opens *one strand* of the duplex at or near *F*, and replication begins. The opened strand is injected progressively into the recipient. As one round of replication and injection is completed, the remaining continuous structure can begin to replicate anew, without significant temporal interruption, yielding a second round of transfer. Since there is physical continuity, linkage of *hfr* and fore markers should be appreciable, and the consequence of the mechanism is continuous transfer.

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SUMMARY

Crosses of donor Hfr Cavalli to a marked F^- recipient were performed under conditions favoring transfer of the complete *E. coli* K-12 linkage group. Genetic analysis of the recombinants revealed that, on completing one round of genetic transfer, donors can initiate a second round in the same order as the first and without a break in the continuity of the linkage group. The continuous linkage group of the donor appears not to open to create a linear structure, with origin

and terminus, but somehow creates an origin while remaining continuous. Linkage of markers in the second round of transfer is much more pronounced than in the first round. The results, confirmed for another Hfr, explain previous reports of linkage across the origin.

Continuous transfer is interpreted on the basis of JACOB and BRENNER's (1963) hypothesis that genetic transfer occurs by DNA replication, with the modification that only one strand of the continuous donor DNA duplex opens upon conjugation, and it is this strand which is replicated into the recipient.

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