THE OCCURRENCE OF A GENETIC TRANSPOSITION IN A STRAIN OF ESCHERICHIA COLI[1,2]

SUSANNE K. DEWITT AND EDWARD A. ADELBERG[3]

Department of Bacteriology, University of California, Berkeley, California

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THE genetic linkage map of *Escherichia coli* strain K-12 has been extensively analyzed. All of the known markers fall into a single linkage group (TAYLOR and ADELBERG 1960); the order of the markers and the distance between them are most readily determined by measuring the time of entry of each marker into the female cell of a conjugating pair, using as the male donor an Hfr strain which transfers its "chromosome" with a specific orientation (JACOB and WOLLMAN 1958).

Hfr strains arise by the mutation of F^+ cells of *E. coli*. Each Hfr mutant transfers its genetic markers in a characteristic sequence. By comparing many different Hfr strains, it is possible to reconstruct the linkage map of the progenitor F^+ strain as a closed curve (Figure 1). Each Hfr is considered to have arisen by the attachment of a formerly autonomous element, the sex factor (F), to the chromosome at a randomly determined site. At conjugation, the chromosome

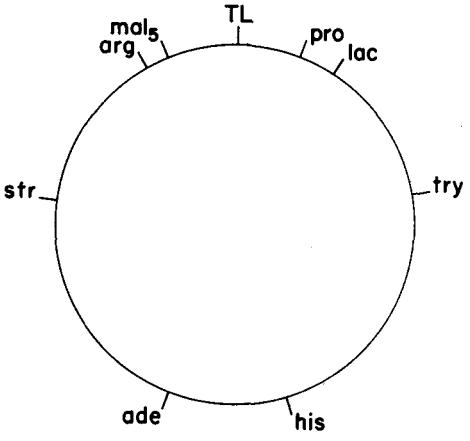

FIGURE 1.—Genetic linkage map of *Escherichia coli* strain K-12. Only the markers used in the investigations reported here are shown. See Table 1 for meanings of symbols.

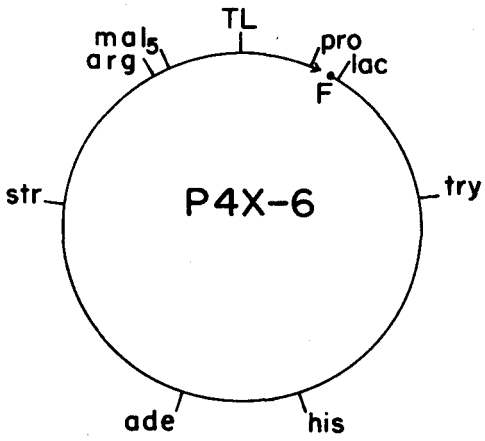

FIGURE 2.—The chromosome of Hfr strain P4X-6, showing the location of origin (→) and of attached sex factor (•—).

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[3] Present address: Department of Microbiology, Yale University, New Haven, Connecticut.

breaks at a point adjacent to the attached F, and the broken end opposite F becomes the leading point, or *origin*, in the transfer process (JACOB and WOLLMAN 1957).

Figure 2 illustrates one such Hfr (strain P4X-6), in which F has become attached between the markers *pro* and *lac* (see Table 1 for the meaning of the genetic symbols). Figure 3 shows the results of a "time of entry" experiment using Hfr P4X-6 as donor. It is seen that the *pro* locus enters at five minutes; TL at 16 minutes; *arg* at 44 minutes; and that the *his* locus is not detected in the recombinants as late as 65 minutes.

In strain P4X-6, F is closely linked to the *lac* locus. In the course of experiments on the phage-mediated cotransduction of lac^+ and F (DEWITT and ADELBERG 1962) a strain was discovered in which a genetic aberration had occurred. The present paper describes the analysis of this aberration, which proved to be the transposition of a chromosomal segment carrying the *his* locus to a new location between the markers *pro* and *lac*. The effect of transferring the aberrant his^+–pro^+ segment into females carrying pro^- and his^- at their normal sites is also described.

MATERIALS AND METHODS

Media and mating conditions: Media for the cultivation of parent cultures and for the selection or scoring of genetic recombinants, as well as the details of the standard conditions for Hfr × F- crosses, have been fully described in a previous communication (ADELBERG and BURNS 1960). Unless otherwise stated, samples of the mating mixtures were plated for recombinant selection at 120 minutes.

TABLE 1

List of strains

	Auxotrophic characters							Energy—source utilization				Response to					
Strain	TL	*pro*	*his*	*try*	*arg*	*met*	*ade*	*lac*	*gal*	mal_1	mal_5	T1	T6	*str*	HT	Hfr_3	Sex
AB735–AB738	+	+	+	+	+	+	+	+	+	+	−	R	R	S	+	+	♂
AB739	+	−	−	−	−	+	+	−	−	+	+	S	R	R	−	−	♂
AB748	+	−	−	−	−	+	+	−	−	+	+	S	R	R	−	−	♀
AB750	+	+	+	+	+	+	+	−	−	+	−	R	S	S	+	+	♂
AB753	+	+	+	−	−	+	+	+	..	..	..	.	R	R	−	..	♂
AB755	+	+	+	−	−	+	+	+	..	..	..	.	R	R	+	..	♂
AB756	+	+	+	−	−	+	+	+	..	..	..	.	R	R	−	..	♂
AB758	+	+	+	−	−	+	+	+	..	..	..	.	R	R	−	..	♂
AB759	+	+	+	−	−	+	+	+	..	..	..	.	R	R	+	..	♂
AB763	−	−	−	+	+	+	+	−	−	−	+	S	R	R	−	−	♀
AB358	+	+	−	+	+	+	−	..	..	−	+	.	S	R	−	−	♀
W3659	+	+	+	+	+	+	+	−	−	+	−	R	S	S	+	+	♀
P4X-6	+	+	+	+	+	−	+	+	+	+	+	S	S	S	−	−	♂
AB332	−	+	−	−	−	+	+	−	−	−	+	R	R	R	−	−	♀
AB531	+	−	−	+	+	+	+	−	+	−	+	R	R	R	−	−	♀
AB1157	−	−	−	+	−	+	+	−	−	+	+	S	R	R	−	−	♀

Symbols: TL, threonine and leucine; *pro*, proline; *his*, histidine; *try*, tryptophan; *arg*, arginine; *met*, methionine; *ade*, adenine; *lac*, lactose; *gal*, galactose; *mal*, maltose; T1, phage T1; T6, phage T6; *str*, streptomycin; S, sensitive; R, resistant; HT, *his* transposition; Hfr_3, sex factor affinity locus; ...., not tested.

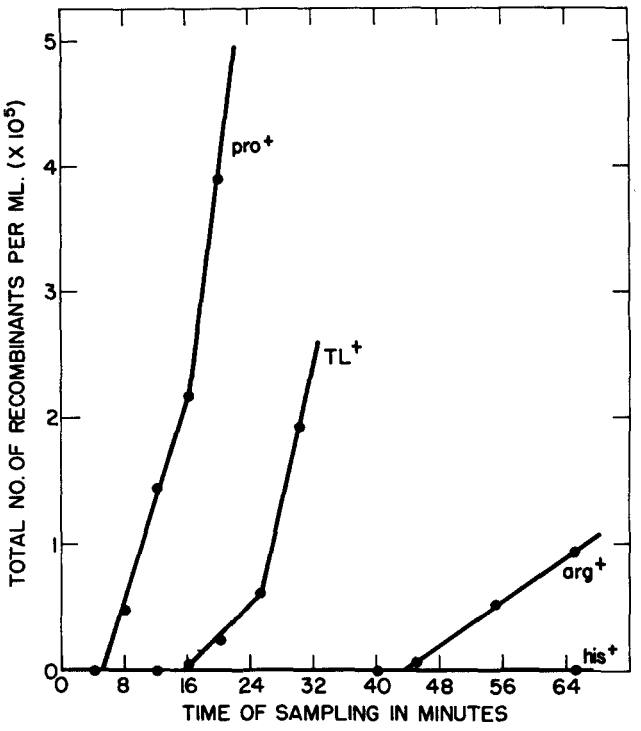

FIGURE 3.—The kinetics of the formation of recombinants in a cross of Hfr strain P4X-6 with female strain AB1157. See the section on Materials and Methods for details of the procedure used.

Strains: The strains referred to in this paper are described in Table 1. They are all originally derived from strain K-12 of *E. coli.*

RESULTS

Evidence for a genetic transposition in certain transduced males: When the *lac*-F segment of the P4X-6 chromosome is transduced by phage into a *normal* K-12 female, the resulting male transductant is an Hfr identical in all respects to its P4X-6 parent (DEWITT and ADELBERG 1962). When such Hfr strains are mated for 120 minutes with a female which is *pro*$^-$–TL$^-$–*arg*$^-$–*his*$^-$–*try*$^-$, the relative frequency with which selected classes of recombinants arise is *pro*$^+$ > TL$^+$ > *arg*$^+$ > *his*$^+$ ≥ *try*$^+$. Some typical data for strain P4X-6 are included in Table 2. This frequency gradient parallels the order of marker transfer shown in Figure 3, and results from the fact that the chromosome undergoes random breakage during transfer with a probability which is constant per unit length.

In one of our transduction experiments, the female strain W3659 was used as the genetic recipient of the *lac*-F segment. The resulting males, when used as donors, exhibited the unique recombination frequency gradient *his*$^+$ > *pro*$^+$ > TL$^+$ > *arg*$^+$ > *try*$^+$ (Table 2). The aberrant state of the *his* marker in four such

TABLE 2

Recombination frequencies for males P4X–6 and AB735

Male	Female	Markers selected from donor					
		his$^+$	*pro*$^+$	TL$^+$	*arg*$^+$	*ade*$^+$	*try*$^+$
P4X–6	AB332	0.002	..	0.7	0.2	..	0.002
AB735	AB332	1.5	..	0.2	0.1	..	0.1
P4X–6	AB531	0.007	2.5	..	..	..	..
AB735	AB531	2.2	0.7	..	..	..	..
P4X–6	AB739	0.008	3.0	..	0.12	..	0.007
AB735	AB739	10.3	3.8	..	1.8	..	0.3
P4X–6	AB1157	0.4	21.0	14.6	3.9	..	..
AB735	AB1157	22.3	9.1	5.1	3.1	..	..
AB735	AB358	20.3	..	..	..	0.9	..

In every case the male parent was contraselected with streptomycin. Recombination frequencies are expressed as the number of recombinants formed per 100 Hfr cells in the mating mixture. The frequencies reported for strain AB735 are typical of those found for all four transduced male derivatives of strain W3659.

males (AB735–738) was further indicated by the fact that when these strains were mated with females and selections made for any male marker other than *his*$^+$, *his*$^+$ appeared as an unselected marker in 95 percent of the recombinants. It was tentatively deduced that in the transduced males the *his* locus lies between *pro* and origin, the time sequence of marker transfer by these males being origin–*his*–*pro*–TL—. This deduction was confirmed by measurements of the time of entry of each marker using the transduced male AB735 as donor. The results (Figure 4) show that this strain transfers *his* at nine minutes, *pro* at 14 minutes, TL at 21 minutes, and *arg* at between 40 and 50 minutes.

The inferred linkage map for strains AB735-738 is shown in Figure 5. The *his* locus is shown to have been transferred to a new location between *pro* and *lac*. The total amount of chromosome transposed takes nine minutes to be transferred (note the displacement of the *pro* locus from a time of entry of five minutes to a time of entry of 14 minutes). This represents approximately eight percent of the total chromosome, which takes 111 minutes to be transferred (TAYLOR and ADELBERG 1960). Strains in which the *his* locus lies between *pro* and *lac* will henceforth be designated HT (for "*his* transposition").

Evidence for a genetic transposition in strain W3659: Since strains AB735–738 all arose by transduction of the *lac*-F segment into female strain W3659, it seemed possible that the observed genetic transposition might already have been present in the latter strain. This suspicion was confirmed by the following experiments.

Strain W3659, provided to us by DR. ALAN RICHTER, carries an *sfa* (sex factor affinity) locus between *mal* and *arg*. RICHTER designated this locus *Hfr*$_3$. When such a strain receives an autonomous F particle by conjugation with an F$^+$ male, the transferred F is attracted to the *sfa* locus and gives rise to both unstable and stable Hfr males. In these males the chromosome breaks so that *arg* is the first marker transferred (RICHTER 1957).

Accordingly, a stable Hfr derivative of strain W3659 was allowed to form by the attachment of F from an F$^+$ donor to the *sfa* locus. The new Hfr, desig-

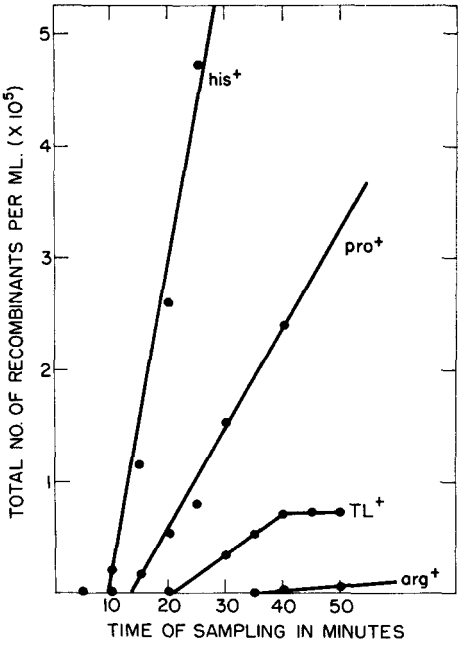

FIGURE 4.—The kinetics of the formation of recombinants in a cross of Hfr strain AB735 with female strain AB1157.

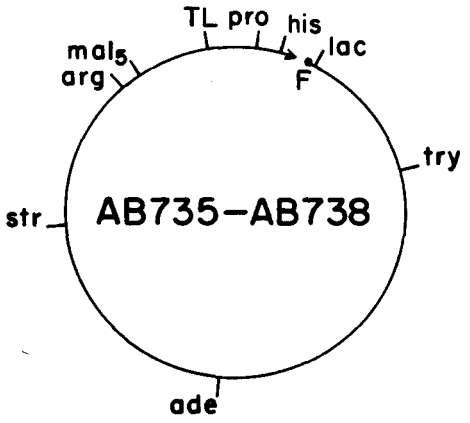

FIGURE 5.—The chromosome of Hfr strains AB735–738.

nated as AB750, was then used as a donor in interrupted mating experiments with an appropriate female. The kinetics of transfer of the *arg*$^+$ marker by strain AB750 are shown in Figure 6. If *his* occupied its normal position in strain AB750, it should have been detected in recombinants starting at about 50 minutes, and should have appeared in a high percentage of *try*$^+$ recombinants selected at 70 minutes (TAYLOR and ADELBERG 1960). Neither was found to be the case: no *his*$^+$ selected recombinants were formed before 95 minutes, and *his*$^+$ did not occur as an unselected marker in any *try*$^+$ recombinants selected at 70 minutes.

When, however, AB750 was allowed to conjugate for 120 minutes, some selected *his*$^+$ recombinants were obtained. Eighty-eight percent of these were found to have received *pro*$^+$ from AB750 as an unselected marker, indicating a close linkage between *his* and *pro* in the latter strain. The linkage map of AB750 can thus be pictured as shown in Figure 7; it differs from that of strain W3659 only in having an attached sex factor between *mal* and *arg*. From these data, and from the data obtained with strains AB735–738, it is concluded that strain W3659 carries a transposition of about eight percent of the chromosome, including the *his* locus.

The fate of the his–pro *segment when transferred to normal females:* In order for a recombinant chromosome to form during the development of a bacterial zygote, it is presumed that pairing must take place between the transferred fragment (exogenote) and the recipient's chromosome (endogenote). When strain

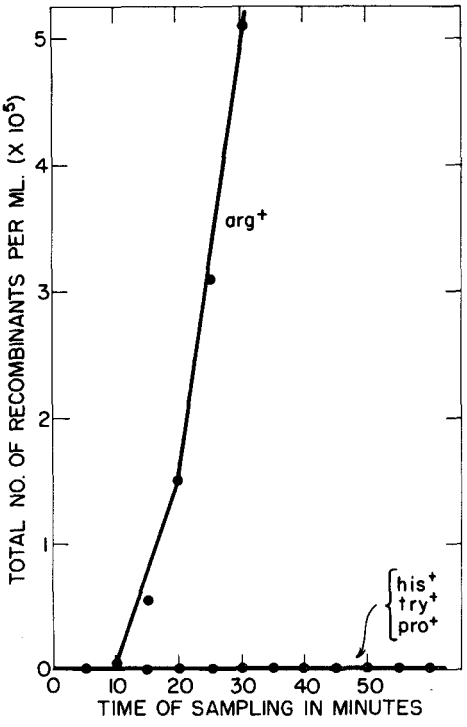

FIGURE 6.—The kinetics of the formation of recombinants in a cross of Hfr strain AB750 with female strain AB739.

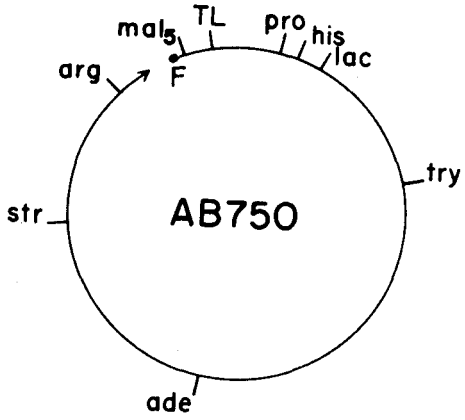

FIGURE 7.—The chromosome of Hfr strain AB750.

AB735 (HT) mates with a normal female, however, the exogenote carries *his*$^+$ and *pro*$^+$ as closely linked markers, while the endogenote carries the corresponding *his*$^-$ and *pro*$^-$ alleles as very widely separated markers (see Figures 2 and 5). The *his*$^+$–*pro*$^+$ recombinants which, as we have seen, arise at high frequency in such a cross, might result from any of four different processes: (1) the *his*$^+$–*pro*$^+$ segment of the exogenote might pair, and ultimately integrate, at the *pro* region of the endogenote; (2) the *his*$^+$–*pro*$^+$ segment might pair, and ultimately integrate, at the *his* region of the endogenote; (3) the markers of the exogenote might separate, or the endogenote deform, allowing the *his*$^+$ and *pro*$^+$ loci to pair with their respective alleles and integrate at their normal locations; or (4) the exogenote might persist and replicate without integration, leading to the formation of a stable, heterozygous, diploid clone.

To discover which of these events occur, it is necessary that the *his*$^+$–*pro*$^+$ recombinants be Hfr males, so that the location of their *his*$^+$ and *pro*$^+$ markers may be determined by observing their transfer in further crosses. Accordingly, strain AB735 (HT) was mated with a *his*$^-$*pro*$^-$*lac*$^-$ female (AB748) and *his*$^+$ *pro*$^+$*lac*$^+$ recombinants obtained. Since F is linked to *lac*$^+$ in strain AB735 (HT), the recombinants were Hfr as expected.

Eight such recombinant males were isolated. The results of testing six of these for their relative order of marker transfer to appropriate females are shown in Table 3. It is seen that the recombinant males fall into two distinct classes: class "A," members of which transfer both *his*$^+$ and *pro*$^+$ before nine minutes, *his*$^+$ giving the highest recombination frequency; and class "B," members of which transfer *pro*$^+$ before nine minutes and at high frequency, while *his*$^+$ is transferred later than 55 minutes and at low frequency. It is inferred that class A recombinant males were formed by an integration of the *his*$^+$–*pro*$^+$ segment of strain AB735 (HT) at the *pro* region of the endogenote, and that class B recombinant males arose by some process which allowed the *his*$^+$ and *pro*$^+$ regions of the exogenote to integrate at widely separated locations, perhaps their normal ones.

The apparent mutational instability of the recombinant males: As mentioned earlier, eight recombinant males were isolated from a cross of Hfr AB735 (HT) with the normal female strain AB748 and purified by repeated single colony isolations. Since we were considering the possibility that some of the recombinant males might represent diploids heterozygous for the *pro* and *his* loci, several hundred colonies of each were grown on a nutritionally complete medium and then replica-plated onto minimal medium to look for *pro*$^-$ and *his*$^-$ segregants. A large number of such colonies (up to 95 percent in some cases) were found to be auxotrophs; when tested, they were found to require one or another of the following growth factors: histidine, proline, uracil, threonine, tyrosine. A mutant unable to use lactose as a carbon source was also detected. It is particularly noteworthy that neither strain AB735 (HT) nor strain AB748 required threonine, uracil, or tyrosine; therefore, segregation of a recessive parental marker was not responsible for the appearance of these mutants. Furthermore, the parental strains appear to be of normal stability, yielding no mutants out of many hundreds of colonies tested.

Since the parental strains differed with respect to their *pro* and *his* alleles, it remained possible that the *pro*$^-$ and *his*$^-$ clones might have arisen from segregants. Several of each type were therefore isolated and purified, and then crossed

TABLE 3

The transfer of genetic markers by male recombinants of strain AB735

Class	Male	Marker selected from donor			Time of entry	
		his$^+$	*pro*$^+$	TL$^+$	*his*$^+$	*pro*$^+$
A	AB755	7.2*	2.2	1.9	< 9′	< 9′
	AB759	12.2*	11.7	11.7	. .	. .
	AB753	4.4	18.8	9.6	. .	. .
B	AB756	1.2	20.0	11.6	>55′	< 9′
	AB758	1.7	35.6	26.2	. .	. .
	AB760	1.1	20.8	. . .	. .	. .

In each cross the male parent was contraselected by the omission of appropriate growth factors. Recombination frequencies are expressed as the number of recombinants formed per 100 Hfr cells in the mating mixture. In every case the female was strain AB763.

* Strains AB755 and AB759 are highly unstable with respect to the *his*$^+$ marker. The *his*$^+$ recombination frequencies for these strains have been expressed relative to the number of *his*$^+$ cells in the Hfr cultures at the time of mating.

with female stocks containing the parental *pro*$^-$ and *his*$^-$ markers, respectively. In every case but one wild-type recombinants were obtained, establishing that the *pro*$^-$ and *his*$^-$ markers found among the recombinant males were not allelic with the markers of AB748, and hence had arisen by mutation rather than by segregation. The *his*$^-$ mutants arose only in the Class A males, in which the *his* locus is in its transposed position.

It is tentatively concluded that the recombinant males are mutationally unstable, in contrast with the parent strains from which they arose. The mutation rates have not yet been measured; they are presumably very high, since it is unlikely that the high proportion of mutants in the populations can be due in every case to a strong selective advantage of the mutants over their parents.

DISCUSSION

The data presented show that strain W3659 carries a genetic transposition of about eight percent of its chromosome. The result is that the *his* locus is no longer in its normal position, but has been inserted between the loci *pro* and *lac*. There is no apparent relationship between the presence of a transposition and the presence of an *sfa* locus in this strain; presumably W3659 had been irradiated several times in order to introduce new markers, so that the two aberrations could have originated independently. Recently, JACOB and WOLLMAN (1961) have reported the occurrence of even larger transpositions following nitrogen mustard treatment.

The mutational instability of recombinants formed when an HT male is crossed with a normal female is entirely unexplained. It seems particularly interesting that the *his* locus is unstable only when it is in its transposed position between origin and *pro*. The genetic determination of instability in these strains will be the subject of future investigations.

SUMMARY

In normal strains of *Escherichia coli* K-12, the *pro* and *lac* loci are very closely linked and the *his* locus is situated about a third of the chromosome away. It has been discovered that the female strain W3659 carries a genetic transposition, such that the *his* locus has been inserted between the loci *pro* and *lac*. The transposed segment represents about eight percent of the chromosome.

Strain W3659 is genetically *lac*$^-$. When phage is used to transduce the *lac*$^+$ marker from the Hfr male P4X-6 into W3659, the attached sex factor of the former is cotransduced. The resultant Hfr males carry the genetic transposition of W3659, and transfer their markers in the order: origin–*his*$^+$–*pro*$^+$—*lac*$^+$-F. One of these, strain AB735, was crossed with a normal female in which the *his*$^-$ and *pro*$^-$ loci are widely separated. Two types of recombinants were formed: type A, in which the *his*$^+$–*pro*$^+$ segment had integrated at the *pro* region; and type B, in which the *his*$^+$ and *pro*$^+$ markers had integrated at widely separated positions. Both type A and type B recombinants appear to be mutationally unstable, but the *his* locus is unstable only when in its transposed position.

ACKNOWLEDGMENT

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