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# Conjugation in *Escherichia coli*: Recombination Events in Terminal Regions of Transferred Deoxyribonucleic Acid

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An investigation of recombination events occurring in zygotes formed during conjugation has been carried out. The frequencies with which donor markers situated close to the origin are recovered indicates an obligatory interaction between the recipient chromosome and a region on the donor chromosome adjacent to the leading end. The partial exclusion from recombinants of some of the most proximal markers studied, however, indicates that this interaction does not occur at the free extremity of the transferred deoxyribonucleic acid. Alternate models to explain these facts are presented.

Two recent papers (5, 11) have reported that, when recombinants are formed as a result of conjugation, certain donor markers which are situated near the leading end of the transferred deoxyribonucleic acid (DNA) are integrated at lower efficiencies than all other donor markers. The papers differ, however, in the estimated size of the region over which this lowered integration coefficient is to be found. Low (11) reported effects extending over the first 5 min of the transferred material (the length of the entire genome being equivalent to 90 min), whereas Glansdorff (5) observed these effects only in markers situated somewhat less than 1 min from the origin. The explanation offered by Low is based on two assumptions, namely (i) that the average crossover frequency per minute is 20% and (ii) that this crossover frequency is constant for all regions of the transferred DNA. Since most zygotes are only partial diploids, crossover events on either side of a given marker are required for its integration. On the basis of the previously mentioned assumptions, markers situated less than 5 min from the origin would be expected to exhibit decreased integration coefficients because of the nonrecovery as recombinants of zygotes in which the proximal crossover failed to occur.

In apparent conflict with the second of these stated assumptions, other workers (15, 19) have concluded that crossover frequency per unit of length is not constant in all regions but is greatly elevated in the region immediately adjacent to the leading end of the transferred DNA. (The unit length used throughout this paper will be a length of DNA which takes 1 min to be transferred by the conjugation process at 37 C). The increase in the frequency is such that it appears that an obligatory interaction occurs between the donor and recipient DNA in this region.

Over other regions of the donor material, with the possible exception of the distal end (4, 19; Pittard and Walker, *unpublished data*), such interactions are not obligatory events but have a certain fixed low probability of occurring. Our results, to be reported in this paper, suggest, as do those of Verhoef and De Haan (19), that in crosses between male and female strains of *Escherichia coli* K-12 such interactions lead, in about 50% of the cases, to joining of the recipient and donor DNA. The mechanism by means of which this integration of donor DNA occurs has not yet been determined, nor has it yet been established what limits the frequency of the interactions between donor and recipient DNA, but it may be due to an inability of the donor material to form hydrogen bonds with the homologous region of the recipient DNA except in regions where some event has separated the double helix or helices to make this possible. Such events may occur preferentially at ends. Whatever the mechanisms may be, the frequencies with which such events occur and give rise to exchanges are referred to as crossover frequencies. A figure that is often quoted for the average crossover frequency is 20% per minute (8, 6, 11). Our results indicate that the average crossover frequency is considerably less than this and also, as previously stated, that the value is not constant over the entire length of the transferred DNA. It is the purpose of this paper to present new data on the relative frequencies of recombination events in the small region adjacent to the leading end of the transferred DNA and to propose a

model that can explain on the one hand, why the cross-over frequency is elevated in this region and on the other, why certain of the most proximally transferred markers have a lower probability of being integrated than any other markers.

## MATERIALS AND METHODS

*Organisms.* The organisms used in this work were all derivatives of *E. coli* K-12, and are described in Table 1. The chromosomal distribution of relevant markers and the points of origin and the direction of transfer of the various male strains used is shown in Fig. 1.

*Media and conditions of mating.* The media, culture methods, and conditions for uninterrupted crosses have been described by Adelberg and Burns (1). Interrupted matings were performed by use of the technique described by Taylor and Thoman (18).

*Scoring unselected markers.* When recombinants were examined for unselected markers, at least 200 single colonies were patched to selective media, incubated overnight, and tested for their inheritance of unselected markers by the replica plating technique. When *tna* was scored as an unselected marker, the patches on the master plate were inoculated into 4-ml quantities of peptone water in Bijou bottles. These were incubated at 37 C for 18 hr and then tested for indole production by adding 2 ml of a 1% solution of *p*-dimethylaminobenzaldehyde in acid alcohol (95 ml of 95% ethyl alcohol and 20 ml of 10 N hydrochloric acid). Those cultures that were *tna*$^+$ gave a characteristic pink color with this reagent.

## RESULTS

The first experiments were concerned with the confirmation of Glansdorff's recent findings and require a short explanation. Pittard and Adelberg (14, 16), examining the relative order of genes on the F-merogenote $F_{14}$ which is carried by F′ strain AB1206, used interrupted mating experiments as the primary method of analysis.

The results of such experiments indicated that strain AB1206 transferred markers in the order *metB1*$^+$, *argH1*$^+$, *ilv-16*$^+$, *F*. [Strain AB1206 has a chromosomal deletion corresponding to the F-merogenote and consequently transfers chromosomal markers at very low frequencies (17)]. When a variety of different Hfr strains were used as donors, however, the relative order of the markers was found always to be *argH1*$^+$, *metB1*$^+$, *ilv-16*$^+$ or *ilv-16*$^+$, *metB1*$^+$, *argH1*$^+$. It was therefore concluded that *argH1*$^+$ had undergone a transposition on $F_{14}$. The kinetic data appeared to be confirmed when *metB1*$^+$ recombinants selected at different times during the mating process were analyzed for their inheritance of *argH1*$^+$ as an unselected marker. The percentage of *metB1*$^+$ recombinants that inherited *argH1*$^+$ increased with time, as expected if *argH1*$^+$ was being transferred distally to *metB1*$^+$. In a more intensive study of the topography of $F_{14}$, Glansdorff (5), using transduction as well as conjugation, has shown that neither *argH*$^+$ nor the closely

TABLE 1. *List of strains*

| Strain no. | Genotype[a] | | | | | | | | | | | | | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *thi* | *ilv* | *met* | *argH*[b] | *argE* | *thr* | *leu* | *pro* | *his* | *mal* | *xyl* | *tna* | *tsx* | *str* | |
| AB1206 | 1[c] | + | + | + | + | + | + | A2 | 4 | + | + | + | + | 8 | ♂ $F_{14}$ |
| AB3308 | − | +/7 | +/+ | +/+ | +/+ | + | + | A2 | + | + | − | + | + | ? | ♂ $F_{14}$ |
| AB2154 | − | + | E47 | + | + | 1 | 6 | + | + | + | + | + | + | 8 | ♂ Hfr |
| AB2918 | ? | + | B1 | + | + | + | + | 351 | + | + | + | + | ? | + | ♂ Hfr |
| AB259 | − | + | + | + | + | + | + | + | + | + | + | + | ? | + | ♂ Hfr |
| AB3311 | ? | + | − | + | + | + | + | + | + | + | + | + | ? | + | ♂ Hfr |
| AB1450 | 2 | 16 | B1 | 1 | + | + | + | + | 1 | 1 | 4 | + | 7 | 9 | ♀ |
| AB2920 | ? | 7 | B1 | + | 3 | + | + | + | 4 | + | − | + | 358 | + | ♀ |
| AB2147 | 2 | 192 | B1 | 1 | + | + | + | + | 1 | 1 | 4 | 1 | 7 | 9 | ♀ |
| AB2915 | 1 | 7 | + | + | 3 | 352 | 351 | A2 | 4 | 358 | − | + | 358 | 704 | ♀ |

[a] The following symbols stand for structural genes concerned with various biosynthetic or catabolic pathways: *thi*, thiamine biosynthesis; *ilv*, isoleucine and valine biosynthesis; *met*, methionine biosynthesis; *arg*, arginine biosynthesis; *thr*, threonine biosynthesis, *leu*, leucine biosynthesis; *pro*, proline biosynthesis; *his*, histidine biosynthesis; *mal*, maltose utilization; *xyl*, xylose utilization; *tna*, tryptophan utilization. The symbol *tsx* stands for a gene determining response to phage T6, and *str* for a gene determining response to streptomycin; the mutant allele denotes resistance to these agents; ?, not tested; +/−, heterozygous.

[b] In keeping with the paper of Glansdorff (5), *argH* represents the structural gene for the enzyme L-argininosuccinate arginine lyase, and *argE* represents the structural gene for the enzyme L-ornithine α-*N*-acetylornithine lyase.

[c] Numbers refer to allele numbers allotted to mutant strains in this laboratory and in the laboratory of E. A. Adelberg.

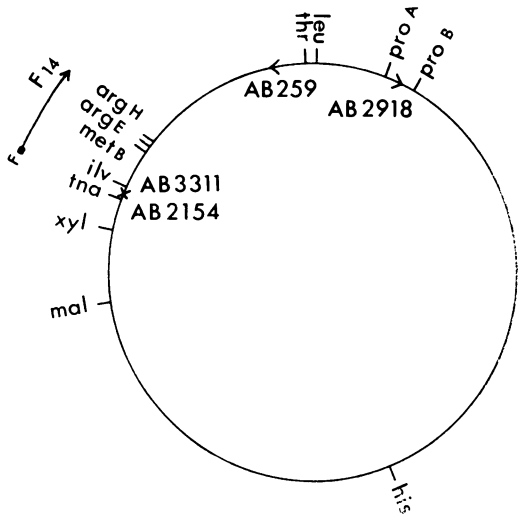


FIG. 1. *Map of the Escherichia coli chromosome showing the points of origin and direction of transfer of the various male strains used, and the location of the genetic markers relevant to this work. For abbreviations, see footnote to Table 1.*

TABLE 2. *Analysis of unselected markers in the mal⁺ recombinants formed in the cross AB2154 × AB2147*

| Unselected marker in selected class | Per cent | Region of proximal crossover (Fig. 5) | Approximate size of region (min) | Crossover frequency per min |
|---|---|---|---|---|
| *xyl⁻* in *mal⁺*..... | 14 | A | 5 | 2.8 |
| *tna⁻* in *xyl⁺ mal⁺*.. | 18 | B | 2 | 9 |
| *tna⁺* in *mal⁺*..... | 72.5 | C | 1 | 72.5 |

TABLE 3. *Analysis of unselected markers in the mal⁺ recombinants formed in the cross AB2918 × AB2147*

| Unselected marker in selected class | Per cent | Region of proximal crossover (Fig. 6) | Approximate size of region (min) | Crossover frequency per min |
|---|---|---|---|---|
| *xyl⁻* in *mal⁺*..... | 9 | A | 5 | 1.8 |
| *tna⁻* in *xyl⁺ mal⁺*. | 2.2 | B | 2 | 1.1 |
| *ilv⁻* in *tna⁺mal⁺*.. | 2.2 | C | 1 | 2.2 |

linked *argE⁺* gene is transposed on $F_{14}$, but that when transferred by this F-merogenote both these genes have decreased integration coefficients relative to other markers, including the nearby *metB1⁺*, which consequently appears to precede them in time of entry experiments. The unselected marker data of Pittard and Adelberg is, in retrospect, readily explained, as with increasing time more and more of the zygotes that had received *metB1⁺* also received the entire F-merogenote including sex-factor. When the entire F-merogenote was transferred, integration was no longer necessary, as the F-merogenote was capable of autonomous replication and, therefore, the loss of *argH1⁺* recombinants during integration no longer occurred. The reported increase of *argH1⁺* recombinants in the *metB1⁺* class in fact reflected the increase in the percentage of *metB1⁺* recombinants that were partial diploids for the entire $F_{14}$ region.

When a first-generation male, which is a partial diploid for the $F_{14}$ region, acts as a donor, the transfer of *metB1⁺* and *argH1⁺* is often linked, by recombination events in the donor, to the transfer of donor chromosomal genes. In these cases, sex factor is not transferred and integration is essential for recombinant formation. Under these circumstances, any effect on the integration of *argH1⁺* is more readily seen. In the case of certain male strains which are very good donors of chromosomal markers and relatively weaker donors of the intact F-merogenote (14), effects on the integration of donor markers is even more apparent. One such strain, AB3308, was used in interrupted mating experiments with the female strains AB1450 (*argH1 metB1 ilv-16*) and AB2920 (*argE3 metB1 ilv-7*). The genotype of the male donor is shown in Fig. 2. The matings were carried out as described in Materials and Methods, and samples were taken every 2.5 min. The results of these experiments (Fig. 3) show that both *argH1⁺* and *argE3⁺* precede *metB1⁺* into the recipient, as suggested by Glansdorff and that the slopes of the curves for the recovery of *argH1⁺* and *argE3⁺* are depressed by comparison with the curve for the recovery of *metB1⁺*. If the *metB1⁺* recombinants selected at different times before the entry of sex-factor are checked for their inheritance of *argH1⁺* in the case of the cross AB3308 × AB1450, and *argE3⁺* in the case of the cross AB3308 × AB2920, these markers are recovered at the constant values of 25 and 35%, respectively (Fig. 4).

These results suggest, then, that the crossover events occurring between *metB1⁺* and origin are distributed so that 65% occur between *metB1⁺* and *argE3⁺* excluding both *argE3⁺* and *argH1⁺* from the recombinants, 10% between *argE3⁺* and *argH1⁺* integrating *argE3⁺* but not *argH1⁺*, and 25% between *argH1⁺* and origin. Unfortunately, we do not have a sufficiently accurate measure of these small regions to determine whether the distribution of crossovers between *metB1⁺* and origin is directly related to the distances involved.

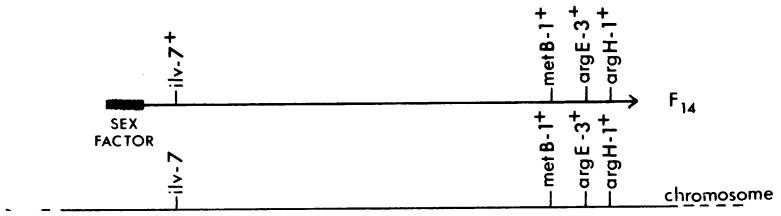


FIG. 2. *Schematic representation of the genes carried by the F-merogenote $F_{14}$ and the homologous region of the chromosome of the F' strain AB3308.*

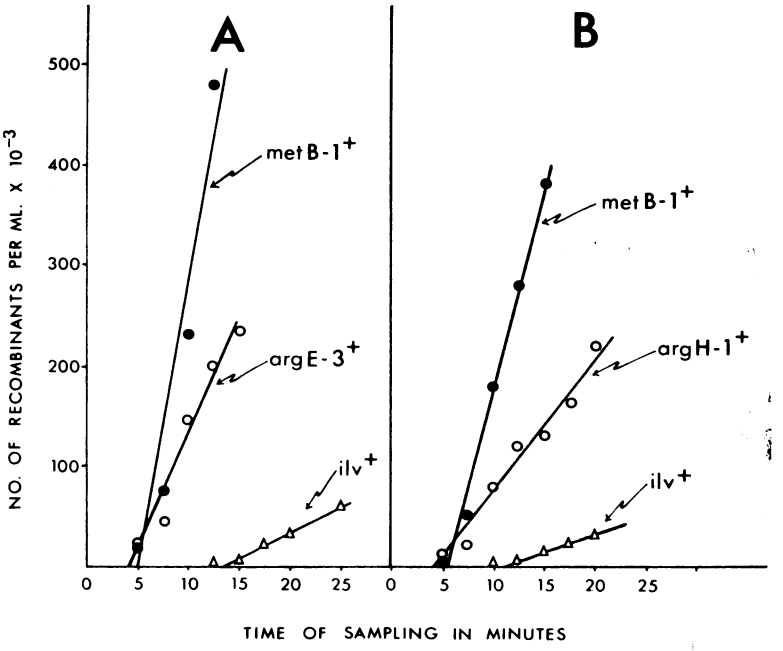


FIG. 3. *Kinetics of zygote formation when the F' strain AB3308 is mated (A) with the recipient AB2920 (argE-3 metB-1 ilv-7) and (B) with the recipient AB1450 (argH-1 metB-1 ilv-16).*

What is known, however, is that if, in such a cross as the one described above, selection is made for a chromosomal marker such as *xyl*$^+$ then approximately 65 to 70% of these recombinants have integrated *metB1*$^+$ and, as expected, a lower percentage (30%) have integrated *argH1*$^+$ (15). This means that the great majority of crossovers have occurred in the small region *metB1*$^+$ to origin (approximately 1 min or less) rather than in the much larger region of *xyl*$^+$ to origin (approximately 8 min). If an even more distal chromosomal marker such as *his*$^+$ is selected, the inheritance of *metB1*$^+$ falls to 45% and that of *argH1*$^+$ to 21%. In other words, the value that is obtained for the inheritance of *metB1*$^+$ is that which would be expected if an obligatory interaction occurred between donor and recipient DNA in the region *metB1*$^+$ to origin and if in approximately 50% of the cases this interaction gave rise to the integration of donor DNA at this point. The higher value of 70% for *metB1*$^+$ as an unselected marker obtained in the case of the *xyl*$^+$ recombinants reflects the fact that only some of the zygotes which fail to integrate the donor DNA in this obligatorily paired region at the origin manage to form a second pairing region somewhere between origin and *xyl*. The failure to recover such zygotes as *xyl*$^+$ recombinants causes an apparent increase in the crossover frequency between *metB1*$^+$ and origin.

A number of other experiments involving different Hfr strains also confirm the fact that the region immediately adjacent to the leading end of the transferred DNA represents a region of greatly elevated crossover frequency. The sex-factor in strain AB2154 is integrated between the closely linked markers *tna* and *ilv* which

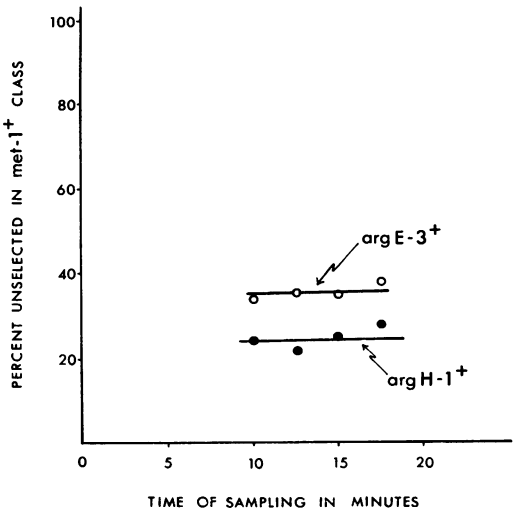


FIG. 4. *Per cent recovery of the unselected donor markers argE-3$^+$ and argH-1$^+$ in recombinants selected for the donor marker metB-1$^+$. The argE-3$^+$ data are from the cross AB3308 × AB2920, and the argH-1$^+$ data from the cross AB3308 × AB1450.*

are approximately 1 min apart (13). Consequently, the gene *tna*, which is transferred as an early marker, must be less than 1 min from the origin of strain AB2154. Hfr AB2918, on the other hand, has sex-factor integrated between *proA* and *proB*, and, although it transfers markers in the same sense as AB2154, the marker *tna* is, in this strain, situated about 25 min from the origin. Both these male strains were mated with the female strain AB2147 (*ilv*$^-$ *tna*$^-$ *xyl*$^-$ *mal*$^-$), and after 90 min samples were plated to select *mal*$^+$ recombinants. These recombinants were scored for *xyl*$^+$ and *tna*$^+$ as unselected markers, as described in Materials and Methods; in the case of AB2918, the inheritance of *ilv*$^+$ was also measured. The genotypes of zygotes giving rise to *mal*$^+$ recombinants in both crosses are shown in Fig. 5 and 6. The distribution of unselected markers in the *mal*$^+$ class was used to determine the number of crossovers per minute occurring in the different regions A, B, and C. The results of this analysis are shown in Tables 2 and 3. Two important facts emerge from a study of these results. The first is that, in the case of the Hfr AB2918, although the average crossover frequency per minute is found to be approximately the same for regions A, B, and C (Fig. 5 and 6), this value of about 2% is much lower than the previously quoted value of 20% (6, 8, 11). The second fact is that, in the case of the Hfr AB2154, the crossover frequency per minute for regions A, B, and C is no longer constant. The value of 2.8% is obtained between *xyl* and *mal*, 9% between *xyl* and *tna*, and 72.5% in the region *tna* to origin. As in the previously reported cross, if recombinants are selected for the more distal marker *his*$^+$ rather than *mal*$^+$ the crossover frequency for the region *tna* to origin is approximately 50%, as expected. It should be pointed out that in both the cases discussed so far the distance between the marker under consideration and origin has been 1 min, thus making the per cent crossovers per region equal to crossover frequency per minute. It can also be seen that, although the greatest elevation of crossover frequency occurs in the region 1 min from origin, some effect is observed as far as 3 min from the origin.

The finding in this experiment of the very high recovery of *tna*$^+$ in the *mal*$^+$ class and of the 50% recovery of *tna*$^+$ in the *his*$^+$ class indicates that the gene *tna*$^+$ is not being integrated with the low efficiency that was observed for *argE*$^+$ and *argH*$^+$ in the previously mentioned crosses. It would seem likely, therefore, that *tna* is located further from the origin of AB2154 than either *argE* or *argH* is from the origin of AB3308. In fact, the behavior of *tna*$^+$ in this cross closely parallels that of *metB1*$^+$ reported in the previous cross. Unfortunately, we do not possess mutant strains with mutations that are located between *tna* and the origin of Hfr AB2154, and we have not therefore been able to show decreased marker integration effects with this particular male. A similar situation applies to the next cross to be discussed.

In this case, experiments similar to those described for AB2154 and AB2918 have also been carried out with Hfr Reeves 1 (AB3311) and Hfr Hayes (AB259) and the female AB2915 (*ilv*$^-$ *arg*$^-$ *thi*$^-$ *thr*$^-$ *leu*$^-$ *pro*$^-$ *his*$^-$). Mating was again carried out for 90 min, and selection was made

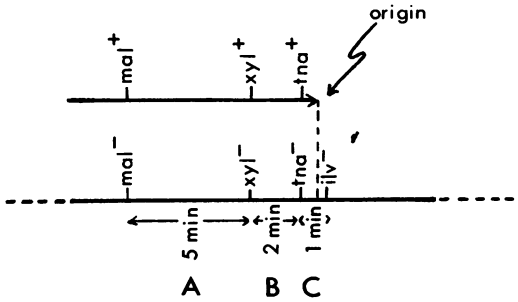


FIG. 5. *Genotype of zygotes giving rise to aml*$^+$ *recombinants in the cross AB2154 × AB2147. Distances in minutes between markers in this and succeeding figures are derived from data of Taylor and Thoman (18) and from experiments conducted in this laboratory.*

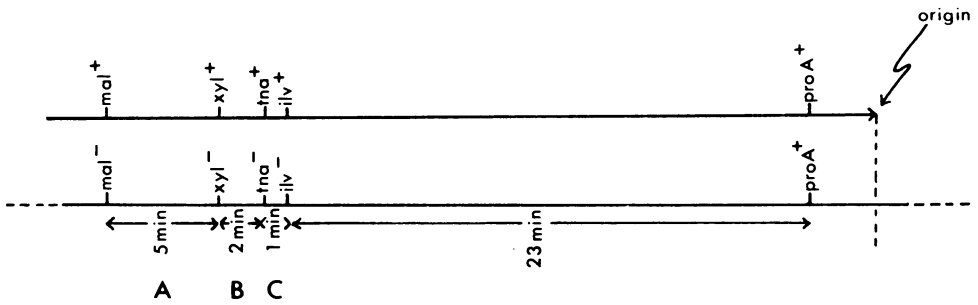


FIG. 6. *Genotype of zygotes giving rise to mal+ recombinants in the cross AB2918 × AB2147.*

for *pro*+ recombinants. These were then scored for their inheritance of *leu*+, *thr*+, and, in the case of the Reeves 1 cross, *arg*+. As in the previous experiment, crossover frequencies per unit of length were then determined and these results are expressed in Tables 4 and 5. The genotypes of the respective zygotes giving rise to the *pro*+ recombinants in each cross are shown in Fig. 7 and 8.

As in the previous cross, the crossover frequency per minute increases in regions adjacent to the leading end. In this case, the figure of 18% is somewhat lower than the 72% recorded for the first set of crosses, but is expected, since *thr* is much further away from the origin of Hfr Hayes than *tna* is from the origin of AB2154. In other words, we would expect that the bulk of crossovers occurring in the region between *thr* and the origin are occurring within the first minute of this region. Although this cannot be shown in these experiments, the assumption is supported by the published data of Lederberg (10).

TABLE 4. *Analysis of unselected markers in the proA+ recombinants formed in the cross AB259 × AB2915*

| Unselected marker in selected class | Per cent | Region of proximal crossover (Fig. 7) | Approximate size of region (min) | Crossover frequency per min |
|---|---|---|---|---|
| *leu*− in *pro*+..... | 19 | A | 6 | 3.1 |
| *thr*− in *pro*+ *leu*+. | 15 | B | 1 | 15 |
| *thr*+ in *pro*+..... | 72 | C | 4 | 18 |

TABLE 5. *Analysis of unselected markers in the proA+ recombinants formed in the cross AB3311 × AB2915*

| Unselected marker in selected class | Per cent | Region of proximal crossover (Fig. 8) | Approximate size of region (min) | Crossover frequency per min |
|---|---|---|---|---|
| *leu*− in *pro*+..... | 11.5 | A | 6 | 1.9 |
| *thr*− in *pro*+ *leu*+.. | 5 | B | 1 | 5 |
| *arg*− in *pro*+ *thr*+.. | 26 | C and D | 11 | 2.4 |

## DISCUSSION

The analysis of unselected markers for the three crosses that have been reported clearly indicates that crossover frequency per unit of length is greatly elevated in a region immediately adjacent to the leading end of the transferred DNA, being most pronounced in a region extending for not more than 1 min from the origin. The frequency of these crossover events suggests that an obligatory interaction between donor and recipient DNA occurs in this region, by contrast with other regions of the chromosome where such interactions have a relatively low probability of occurring.

In the experiments reported in this paper, approximately 50% of the interactions which occurred in this most proximal region resulted in a recombination event joining donor and recipient strands. Our results suggest that markers that are as close as 1 min from the origin do not consequently suffer a decreased probability of integration due to the nonoccurrence of a proximal crossover event. Our conclusions therefore differ markedly from those of Low (11), who suggested that such integration effects would occur with markers located as far as 4 or 5 min away from the origin.

Curtiss (3) recently postulated that pairing between the leading end of the donor DNA and the homologous region of the recipient chromosome is essential for DNA transfer. He suggested that processes which occur in the recipient cell effectively pull the donor DNA across the conjugation bridge. Whether one of these processes involves the replication of the recipient chromosome is currently a matter of disagreement (2, 3). Johnson, Falkow, and Baron (9) have also demonstrated the importance of homology between the proximal region of transferred DNA and the

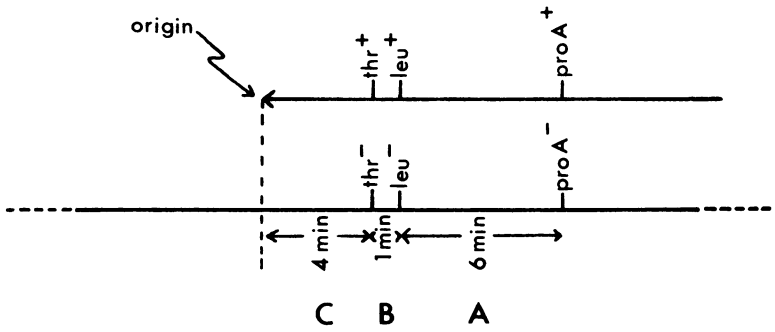


FIG. 7. *Genotype of zygotes giving rise to proA+ recombinants in the cross AB259 × AB2915.*

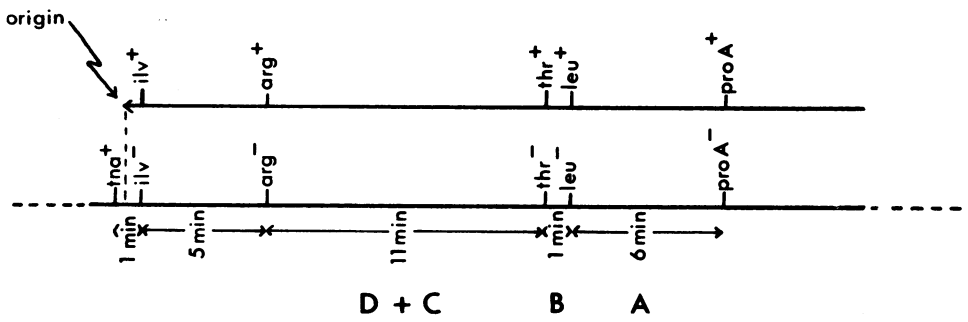


FIG. 8. *Genotype of zygotes giving rise to proA+ recombinants in the cross AB3311 × AB2915.*

recipient chromosome in a study of conjugation between *Escherichia coli* and *Salmonella typhosa*.

Although our results suggest that in recombinant formation an obligatory interaction occurs between donor and recipient DNA at a region adjacent to the leading end, they do not indicate whether such an interaction is essential for the transfer or for the integration of donor DNA.

The fact that certain of the most proximal markers to be transferred (e.g., *argE* and *argH* in the case of the F′ crosses) are integrated at a relatively lower frequency than other markers indicates clearly that the recombination events occurring in this proximal region do not occur preferentially at the free end of the transferred DNA. The simplest explanation for the low recovery of these markers is that crossover events are distributed randomly within this region of obligatory interaction. In this case, markers very close to the proximal end of this paired region would have a certain probability of being excluded from recombinants. Such a possibility has been proposed by Glansdorff (5). A quantitative confirmation of this theory, however, requires a more accurate knowledge of the relative positions of these early markers with regard to the origin and a more accurate measure of the size of effectively paired regions between donor and recipient DNA (12, 15).

Another explanation which can be offered to account for the phenomenon of lowered recovery of very early markers depends on whether or not a small part of the sex factor forms the leading end of the transferred DNA. This hypothesis is not new, as it is implicit in the replicon theory of Jacob, Brenner, and Cuzin (7), and in fact the presence of an origin factor at the leading end of transferred DNA has previously been proposed by Adelberg and Burns (1). It has already been demonstrated in transduction experiments that the presence of sex-factor DNA on donor fragments interferes with the integration of these fragments when introduced into female strains, by creating a region of nonhomology between recipient and donor strands (13). It is possible that, in a similar fashion, sex-factor DNA, present at the leading end of the donor DNA, may also exert an antipairing effect in zygotes. In this model, therefore, exclusion of certain markers from recombinants would not depend on the random distribution of crossover events in the proximal effectively paired region, but would be due to the exclusion of such markers from this region caused by the antipairing effect of the closely linked sex-factor DNA. A prediction that can be made if this latter theory is correct is that, if an Hfr which is isogenic with the donor strain with regard to sex-factor attachment is used as a recipient, then

no lowering of integration efficiencies of the most proximal markers should be observed. Unfortunately, this possibility cannot readily be testcd in the system described in this paper.

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