DELETIONS IN LIMITED HOMOLOGY RECOMBINATION IN *ESCHERICHIA COLI*

DAVID P. FAN[1]

MRC Laboratory Molecular Biology, Hills Road, Cambridge, England

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AN F episome in the bacterium *Escherichia coli* can replicate autonomously or become integrated into the chromosome by genetic recombination to form Hfr strains. In these latter strains the multiplication of episomal genes is under chromosomal control. If the episome and chromosome carry allelic genes, then there are large regions of homology at which integration usually occurs (CUZIN and JACOB 1964; PITTARD and RAMAKRISHNAN 1964). Otherwise, integration still can take place but only between episomal and chromosomal sites which are limited in homology (SCAIFE 1966; BERG and CURTISS 1967).

In order to study limited homology recombination, experiments were performed on the strain W3747 (HIROTA and SNEATH 1961) which carries F13 and is deleted in the chromosome for all the genes on F13. This episome contains genes indispensable for cell growth; therefore, it cannot be lost. However, growth in the presence of acridine orange permits chromosomal replication while blocking F13 replication. Hence the only way W3747 can grow is for F13 to become integrated into the chromosome using regions limited in homology (SCAIFE 1966).

The mechanism of this integration has already been presented in broad outlines (SCAIFE 1966, BERG and CURTISS 1967). In this mechanism, there is a break in the episome and in the chromosome after which chromosomal ends are joined with episomal ends. In the simplest detailed model, there is a reciprocal recombinational event with no loss of DNA but only a rearrangement. Figure 1 illustrates this model for the case when F13 is disrupted in the middle of the *z* gene of *lac* and integrated between *trp* and *his*. The end result is that the two ends of the *z* gene are separated by the distance of the chromosome. The strong prediction of this model is that in each integration event only one episomal gene can be disrupted. In fact it will be shown in this communication that neighboring genes are also disrupted indicating that deletions are created around the regions where limited homology integration occurs.

MATERIALS AND METHODS

Bacterial strains (all from the Cambridge Collection, MRC Laboratory of Molecular Biology, Cambridge, England):

W3747—F13/*met*$^-$*T6*^{r}Δ*F13* where the Δ*F13* refers to the fact that all chromosomal genes on F13 are now deleted from the chromosome (see Figure 1, the strain was initially described

[1] Present address: Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55101.

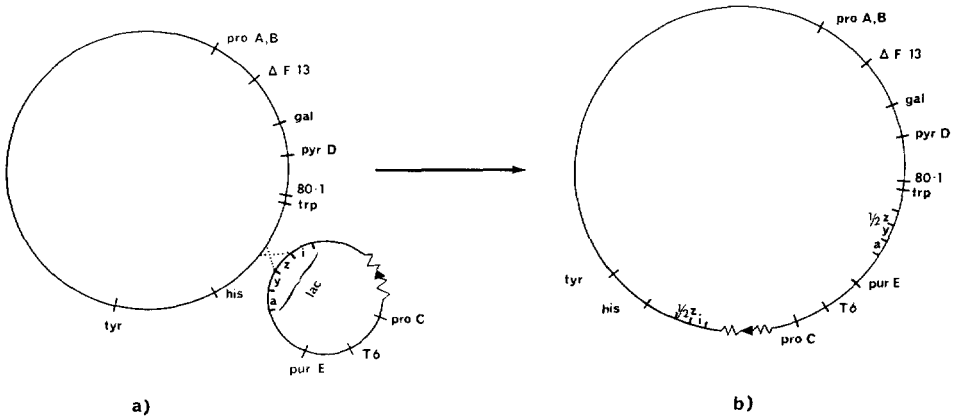

Fig. 1

FIGURE 1.—Reciprocal recombination model for integration of F13 into the chromosome. The partner sites involve the episomal *lacz* region and the chromosomal region between *his* and *trp* (a) Original W3747 strain with *F* genes indicated by the zig-zag line. Integration region indicated by dotted lines. (b) Hfr resulting from the integration event.

by HIROTA and SNEATH (1961) and the detailed map in Figure 1 is from SCAIFE (1966) and D. E. BERG, personal communication).

E56—W3747 with F13 integrated into the chromosome to give an Hfr with donation order *O-lac-tyr-his-pyrD* . . . , isolated by picking and purifying a white colony from W3747 plated on LZAO plates. This strain is of the type described in the second paragraph of the introductory section.

X121—F⁻*tyr*⁻*his*⁻*trp*⁻*pyrD*⁻λ^r*str*^{r}*thi*⁻

X13 —F*lac*+/*arg*⁻*his*⁻*dap*⁻*str*^{r}Δ*lac*

X87 —F⁻*lacO*$_2$⁻*ura*⁻*trp*⁻*his*⁻*str*^{r}

X192—F*lacO*$_2$⁻/*lacO*$_2$⁻*ura*⁻*trp*⁻*his*⁻*str*^{r}

M263—F⁻*lac200*⁻*his*⁻*cys*⁻*str*^{r}

M264—F*lac200*⁻/*lac200*⁻*his*⁻*cys*⁻*str*^{r}

M203—F⁻*lac659*⁻*trp*⁻*str*^{r}

M208—F*lac659*⁻/*lac659*⁻*trp*⁻*str*^{r}

The F*lac* which appears in the above strains has been described by CUZIN and JACOB (1964).

Phage strains (from the Cambridge Collection):

80.1—temperate coliphage

80.1c25—a clear mutant of 80.1

80.1d$_1$*lac* i_3^-—defective 80.1 carrying the *lac* structural genes and the *lac i* gene with the mutation i_3^- (kindly provided by DR. W. EPSTEIN, see SIGNER and BECKWITH (1966) for 80.1d$_1$*lac*).

f2—male specific coliphage

Media: The minimal M9 medium and the Penassay broth were as described by SIGNER (1966). LZ plates contained 25.5 g antibiotic medium (Difco) and 50 mg triphenyl-tetrazolium chloride in 950 ml H_2O (SIGNER, BECKWITH and BRENNER 1965). After autoclaving and cooling to 50°C, 50 ml 20% lactose (autoclaved separately) was added. LZAO plates are the same as LZ except that when the lactose was added, 3 ml 2N NaOH and 2.5 ml acridine orange 10 mg/ml were also added. Lac+ colonies are white and lac⁻ colonies are red on both LZ and LZAO plates.

Transduction: The *lac* genes were introduced into *lac*⁻ bacteria by transduction with an HFT lysate from a bacterial strain lysogenic for 80.1 and 801.d$_1$*lac* i_3^-. Aliquots of this HFT lysate containing about 4×10^8 plaque forming (80.1) particles were spread on lactose selective plates

and about 5 μl of overnight *lac*⁻ bacteria grown in Penassay broth was spotted on the plates which were then incubated at 42°C.

Bacterial crosses on plates (spot crosses): Overnight cultures of donor and recipient bacterial strains were grown in non-aerated Penassay cultures at 37°C. Then 0.25 ml of the recipient was spread on selective plates. After drying, about 5 μl of the donor was spotted on the plate which was incubated at 37°C. Donation of terminal as well as initial markers could be detected easily in Hfr × F⁻ crosses.

Interrupted mating: Donor and recipient strains were both grown to about 3×10^8 in Penassay broth without aeration. Equal volumes were mixed for 5 min and then diluted at least ten-fold into M9 medium containing supplements necessary for the recipient to grow. At various times samples were taken and agitated on a vortex mixer for 20 sec before dilution and plating on selective plates.

Enzyme assays: β-galactosidase as described by NAKADA and MAGASANIK (1964), β-galactoside transacetylase as described by SAMBROOK, FAN and BRENNER (1967).

Abbreviations for genetic markers: arg = arginine; *cys* = cysteine; *dap* = diaminopimelic acid; *gal* = galactose; *his* = histidine; *lac* = lactose, with component genes *i* = repressor, *z* = β-galactosidase, *y* = β-galactoside permease and *a* = β-galactoside transacetylase; *met* = methionine; *pro* = proline; *pur* = purine; *pyr* = pyrimidine; *thi* = thiamine; *trp* = tryptophan; *tyr* = tyrosine; *str* = streptomycin; *ura* = uracil; *80.1* = phage 80.1 receptor identical with one of the receptors for phage T1; *T6* = phage T6 receptor; ^{r} superscript denotes resistance. Abbreviation for chemicals: IPTG = isopropylthio-β,d-galactoside.

RESULTS

In order to see if the acridine orange in LZAO plates will block F episome replication and hence promote integration of the indispensible F13 in W3747, control experiments were first performed on bacteria carrying dispensible F factors e.g. F*lac*⁺. In these experiments, acridine orange treatment should simply lead to loss of the episome. Thus, F*lac*/Δ*lac* bacteria were plated on LZAO to give individual colonies. If the acridine orange in LZAO has no effect, then the F*lac*⁺ would remain in the cell leading to white lac⁺ colonies; however, if the LZAO plates inhibited F episome replication, then the F*lac*⁺ would be lost, giving rise to F⁻Δ*lac* bacteria which are lac⁻ and red. Indeed, most of the colonies are red but a few are white. After prolonged incubation the red colonies contain white sectors. This result indicates that F*lac*⁺ replication is inhibited by acridine orange, but that after further incubation inhibition is less efficient hence permitting the growth of F*lac*⁺ containing bacteria. This finding is not surprising since HIROTA (1960) has already found that when the cell density is too high or when the pH is too low curing is inefficient. As the colonies grow on LZAO plates, the cell density certainly increases and the pH may well drop in the vicinity of the colonies. The LZAO plates may have a slightly lethal effect on bacteria because when E56 is plated on these plates the number of colonies which appear is about ⅓ to ½ of that on LZ plates.

The principal experimental approach used to study limited homology integration was first to treat W3747 with acridine orange to obtain strains in which the F13 episome had integrated into the chromosome and then to analyse genetically the resulting strains. In order to obtain a large number of strains arising from independent integration events, the acridine orange treatment was performed by plating W3747 cells to give individual colonies on LZAO plates (about 1000 per

plate). Since acridine treatment occurred only on the LZAO plates, each colony which arose should contain integration events independent of those in other colonies. As expected, most of the colonies which arose were white, indicating that the bacteria in them were still *lac*$^+$. However, about one in 500 to 1000 colonies contained a red sector suggesting that *lac*$^-$ bacteria were present. The frequency of red-sectored colonies decreased greatly if the colonies became too crowded so that at 10^4 colonies per plate, no red sectors were found in about 10^5 bacteria plated. Also no red colonies appeared in 2×10^4 colonies if the acridine orange was omitted from the plates. Since the frequency of the occurrence of the red-sectored colonies is very high under optimal conditions, the most likely interpretation for them is that they contained bacteria in which integration to form Hfr's took place with the episomal partner site being in *lac* (e.g. Figure 1). It is unlikely that LZAO plates induced mutation to *lac*$^-$ with such a high frequency since E56 which is already Hfr but contains essentially the same genetic information as W3747 does not give any red-sectored colonies in 2×10^4 screened.

Completely red colonies were about one-half as frequent as red sectored colonies. This result suggests that the lac$^+$ F13 parent grew very slowly; then several different integration events took place giving rise to different strains all of which could grow more rapidly than the parent. These progeny would form the bulk of the colony. Statistically, it would be expected that some colonies would contain the progeny of a single integration event since integration occurs only rarely. Almost all of the W3747 cells plated must have yielded at least one progeny all derived from an integration event because W3747 also gives about ⅓ as many colonies on LZAO as on LZ.

Because the genetic region in and around *lac* is well marked, it was decided to study primarily those integration events in which the *lac* gene had been disrupted. Thus lac$^-$ bacteria were isolated from 195 different red-sectored colonies and their properties analysed (Table 1). Only three of the tests used need be described in detail.

The presence of the *i* gene in the *z*$^-$ isolates was determined by transducing the isolates with 80.1d$_i$*laci*$_3^-$. Since this phage does not carry a functional *i* gene, lysogenization with this phage will lead to constitutive β-galactosidase synthesis from the *z* gene carried by the phage unless the bacterium can provide an active *i* gene product in which case β-galactosidase synthesis will be inducible. Thus lysogenized isolates were grown in Penassay broth without inducer and β-galactosidase measured to see if it is constitutive. Cultures induced with 5×10^{-4} M IPTG were used to test if synthesis is inducible.

The absence of the *F* genes makes a strain F$^-$. The F$^-$ character was established for the isolates of class B3 by their inability to donate the markers *his* or *trp* when spot crossed with the F$^-$ bacteria X87, M203, or M263; their inability to grow the F specific phage f2; and their ability to accept with high frequency F*lac*$^+$ from X13. Furthermore the F*lac*$^+$ could be cured easily on LZAO plates as described in the first paragraph of the RESULTS section. All other isolates contained the *F* genes because they could donate the *his* and *trp* genes in the spot cross tests used above.

TABLE 1

Properties of isolates from E. coli *W3747 in which F13 has been integrated into the chromosome to give red colonies on LZAO plates*

Class	Number of isolates	lac	glu	mel	Reversion	Prototroph	*z* gene	*i* gene	*F* genes	Contains *z* gene fragments
A1	4	+	—	..	..	+	+	..	+	+
A2	5	+	+	..	..	+	+	..	+	+
B1	1	—	+	+	0/1	+	—	+	+	+
B2	168	—	+	—	0/32	+	—	—	+	—
B3	17	—	+	—	0/2	+	—	—	—	—

lac refers to ability to grow using lactose as the carbon source. The cells were spotted in minimal plates containing glucose and methionine.

glu determined for glucose analogously to lac.

mel determined for melibiose analogously to lac.

Reversion refers to the ratio of strains which will revert to *lac*+ to the total number tested. About 10^9 cells were spread on a minimal lactose methionine plate for each isolate tested.

Prototroph refers to the ability to grow on minimal plates using the same supplement, methionine, as the parent W3747.

z gene refers to the presence of a functional *z* gene and is measured by ability to produce β-galactosidase when grown in Penassay broth containing 5×10^{-4} M IPTG.

i gene refers to the presence of a functional *i* gene (see text).

F genes refers to the presence of genes necessary for donation of genetic information, exclusion of F episomes and adsorption of f2 (see text).

Contains *z* gene fragments refers to the fact that the isolates will recombine with *z* point mutations O_2, *200* and *659* to yield *z*+ strains (see text).

In order to test for the presence of all or part of the *z* gene, the Hfr isolates were spot crossed with X87, M263 and M203 to see if they could donate any of the *lacz* O_2, *200* or *659*. The F⁻ isolates were spot crossed with strains X192, M264 and M208 which carry the same mutations on F*lac*. Here, the episome would enter the F⁻ cells and recombine with any *lac* regions in the F⁻ bacteria. In the experiments performed, it was found that an isolate could either recombine with all or none of the *lac* mutations. These test point mutations were used because O_2 maps very close to one end and *659* very close to the other end of the *z* gene while *200* maps about two-thirds of the way in between (NEWTON, BECKWITH, ZIPSER and BRENNER 1965).

Isolates of classes A1 and A2 were not characterized further since they were still *lac*+. These cells presumably have some lesion outside the *lac* locus which leads to red colonies on LZAO and LZ plates. The classes A1 and A2 differ only in that the isolates of class A1 cannot use glucose. These might be permeability mutants unable to transport glucose into the cell.

If an isolate could grow on melibiose as the carbon source, then it would possess an active *y* gene (BECKWITH 1963). This finding would indicate that the *y* gene was intact. However, an isolate can fail to grow on melibiose and yet still contain a complete *y* gene. It is possible, for example in the model given in Figure 1, that *y* was connected to an operon that was not being expressed under the growth conditions used or to an operon in which the direction of transcription was different from that in the *y* gene.

From Table 1 it can be seen that only one z^- isolate (class B1) is i^+, y^+ and contains z gene fragments. This is the only isolate which conforms to the predictions of a model such as is given in Figure 1. However, this isolate contains a z^- mutation which was formed independently of the integration event because it will make β-galactoside transacetylase only on induction with 5×10^{-4} M IPTG. Thus the transacetylase must still be linked directly to the lactose control region and not separated from it by the distance of the chromosome as would be predicted if integration had occurred in the z gene. In interrupted mating experiments with X87 the first marker donated is $lacO_2$ indicating that the episomal partner site for the integration was between *lac* and the *F* genes. This z^- mutation may have been induced by acridine orange; but only one was found in about 2×10^5 colonies examined.

The spot cross experiments indicate that when the z gene is destroyed during integration (classes B2 and B3), practically all the information of the z gene is deleted because none of the isolates produce z^+ recombinants when crossed with the mutants O_2, *200* or *659*. Furthermore, the i gene is also lost and about 10% of the time the *F* genes are lost in addition. Thus the deletion can be quite extensive. As expected, the isolates do not revert to lac^+. CURTISS (1964) has described a strain in which the *lac* gene is disrupted by recombination of an F factor into the z gene. In this strain some of the z gene may also have been lost because the strain will recombine with some but not all z point mutations.

If the partner chromosomal site for the integration had been at a gene responsible for an auxotropic marker then the precise position of integration could be

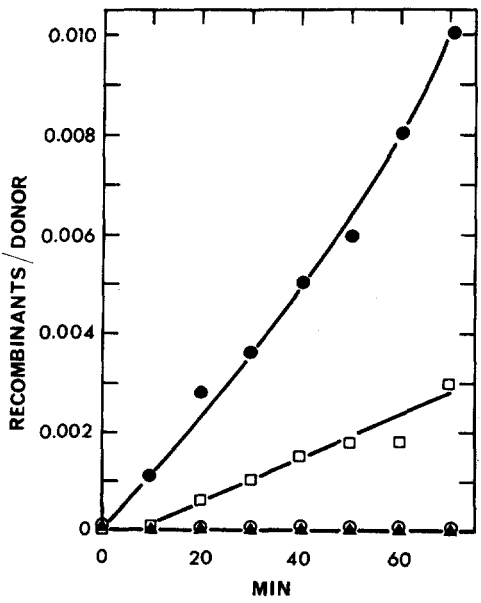

FIGURE 2.—Interrupted mating of X121 with an isolate of class B2. ● – – ●, *trp*; □ – – □, *pyrD*; ▲ – – ▲, *his*; ○ – – ○, *tyr*.

determined by identifying the auxotropic marker which was destroyed. But it can be seen from Table 1 that no auxotrophs were found among the isolates. However, some of the isolates were examined to locate approximately the chromosomal regions involved by interrupted mating experiments. Figure 2 shows a typical experiment for an isolate from class B2. These kinetics were indistinguishable from those shown by 5 other isolates from the same class. F*lac*$^+$ was introduced into the F$^-$ isolates from class B3. These were then used as donors in interrupted mating experiments. If F*lac*$^+$ can find regions of good homology with chromosomal genes, there will be a defined order of entry of markers with origin at the site of good homology with the chromosome (CUZIN and JACOB 1964; PITTARD and RAMAKRISHNAN 1964). Any chromosomal genes in these new F$^-$ strains which could be homologous to F*lac*$^+$ must have come from F13 as that episome covers the entire F*lac*$^+$ region. Thus the origin of donation by F*lac*$^+$ would reflect the point of integration of F13. Figure 3 shows a typical experiment; as expected, the frequency of recombinants is lower than in Hfr donation (Figure 2), but there clearly is a defined order of entry. Three other isolates from class B3 were tested and gave identical marker transfer order. The results of the interrupted mating experiments are given in Table 2 and suggest that there are preferred sites for limited homology integration of F episomes confirming published results (BERG and CURTISS 1967).

It can be asked whether there are simply weak points in the chromosome and episome at which breakage and, hence, recombination can occur more readily or

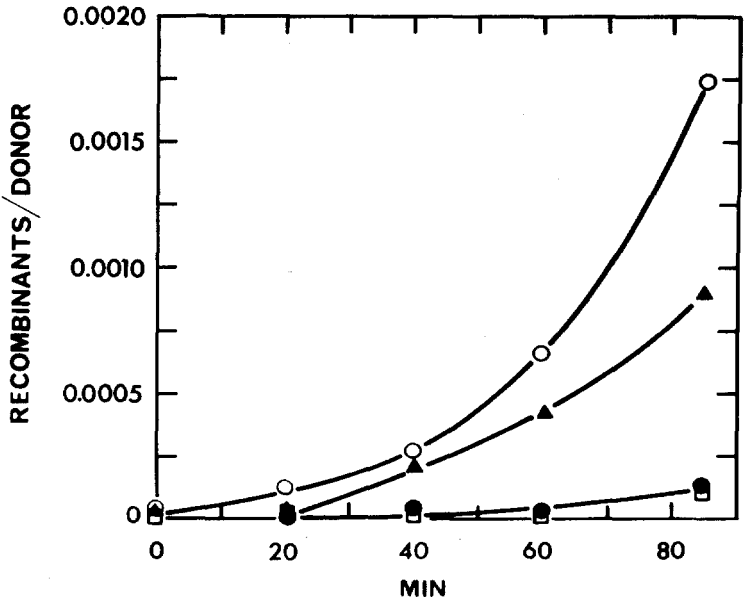

FIGURE 3.—Interrupted mating of X121 with an isolate of class B3 carrying F*lac*+. Symbols as in Figure 2.

TABLE 2

Donation order of chromosomal genes by isolates of Table 1

Class	Number	Donation order
B1	1	*O–his–trp–pyrD . . . tyr*
B2	6	*O–trp–pyrD . . . tyr–his*
	1	*O–tyr–his–trp–pyrD . . .*
B3	4	*tyr–his–trp–pyrD . . .*

Class as defined in Table 1.
Number refers to number found with the order listed; the rest of the mutants of each class were not tested.
For classes B1 and B2 the isolates were crossed with X121 in interrupted mating experiments.
For class B3 the isolates containing F*lac*+ were crossed with X211 in interrupted mating experiments.

whether some homology is required to determine integration sites. The latter hypothesis is valid as seen in experiments using the clear mutant $80.1c_{25}$. About 2×10^8 W3747 cells were plated together with about 10^9 $80.1c_{25}$ on each of 50 ZLAO plates to select bacteria which have integrated F13 and simultaneously become 80.1 resistant. Under these conditions each plate gave about 200 colonies. If integration and production of phage resistance were independent events or if integration were independent of homology, then the appearance of red lac$^-$ colonies should remain one in $5\text{-}10 \times 10^5$. However, in about 10^4 colonies examined, no red colonies were found. This result is most consistent with the interpretation that when the 80.1 resistance site is disrupted by integration of F13, homology selects a partner site on the episome which does not involve *lac*.

DISCUSSION

When F13 is integrated into the chromosome of W3747 destroying the *z* gene, various amounts of genetic information seem to be deleted in the region of the integration. The ends of the deletions seem to depend on the integration site because the integration site seems to be predominantly between *trp* and *his* in those deletions which include *i* and *z* but not the *F* genes while the site is close to *tyr* in those cases in which the *F* genes are also lost. In turn, the integration site is determined by homology as the phage resistance experiments show. Therefore, homology fixes the ends of the deletion.

No measurements were made as to whether in addition regions of the chromosome were deleted during integration. But it can be seen that such deletions probably occur if it is assumed that in the recombination event the cell treats the partners as simply two pieces of genetic information. In this case no distinction will be made as to which partner came from the episome and which from the chromosome. Therefore, chromosomal genes may be deleted about as often as episomal genes which means that in practically every integration event there is a chromosomal as well as an episomal deletion.

Since F episome creation seems to proceed by limited homology recombination in a way analogous to integration (SCAIFE 1966; BERG and CURTISS 1967), it

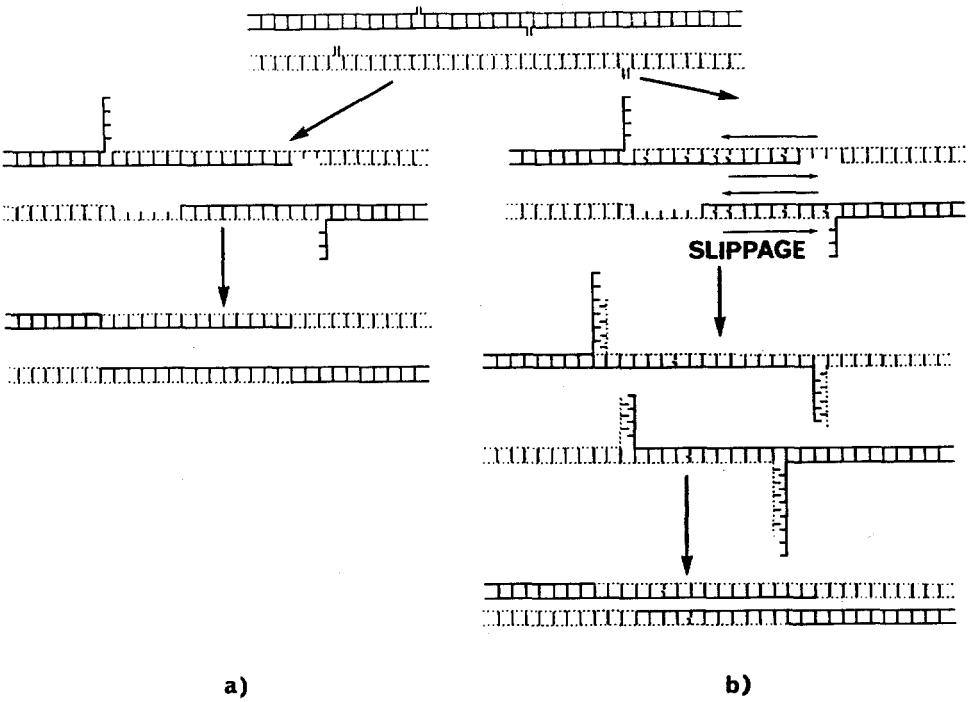

FIGURE 4.—Model for genetic recombination. The short parallel lines indicate bases on the DNA. When they do not cross from strand to strand, there is no hydrogen bond complementarity. (a) Conditions of good homology. Single strand breaks are made in homologous DNA regions in non-coincidental positions. There is a separation then a reannealing of complementary regions to form recombinant hydrogen bonded duplexes. The loose ends are trimmed and gaps filled to form recombinant covalently linked molecules. (b) Conditions of limited homology. Formation of hydrogen bonded duplexes as in (a). But these are unstable because complementarity is not complete. There is a slippage to get better homology. Loose ends are trimmed and backbones joined.

seems likely that reintegration of F episomes in such strains as W3747 would almost never proceed at their original sites (SCAIFE 1966) as the regions of homology used for creation of the episome would have been deleted by the recombination event giving rise to the episome.

Studying the production of deletions and low frequency transducing phages, FRANKLIN (1967) has found that limited homology recombination proceeds normally in the presence of the recombination deficient mutation *recA*$^-$. When this mutation is present, the bacteria seem unable to terminate DNA breakdown and initiate repair synthesis correctly following single strand interruption (HOWARD-FLANDERS and THERIOT 1966).

The model of recombination given by THOMAS (1966) can be readily modified to account for the above data (Figure 4). The sequence of events is the same for both good homology and limited homology recombination up to the point of formation of the annealed hybrid molecules. In good homology recombination the

annealed overlap region is stable enough to maintain the structure while repair enzymes complete the job of filling gaps, trimming ends and then attaching one DNA backbone to another. If the homology is poor, however, there will be slippage in the annealed overlap region until better homology is found at which point excess ends are trimmed off and the backbones joined. In this case, genetic information is deleted in the slipping and trimming processes. Furthermore, the backbones can be joined after a trimming process alone without any repair synthesis thus allowing limited homology recombination to proceed in the presence of *recA*$^-$.

This model is but one of several possible ones which could explain the data. In a modification of this model, the hydrogen bonded duplexes could separate before joining of the backbones. The loose DNA ends would then be degraded before more complementary base sequences were found, the strands reannealed and backbones rejoined. In any model it seems surprising that the deletions which seem to have been found in isolates of classes B2 and B3 (Table 1) should be so great as to include *z*, *i* and sometimes even *F*. In both models presented, it would also be expected that there could be different probabilities with which deletions occur during recombination. If homology were good, then deletions would occur very rarely. If homology were slightly worse, then deletions would arise only some of the time in which case they might be large with ends fixed by new homologies. Or, if homology were very poor, then almost invariably genetic material would be be deleted. In any case, there is no necessary connection between the probability that a deletion occurs and the position of the ends.

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SUMMARY

When an F episome is integrated into the chromosome of *E. coli* by recombination between DNA strands having limited homology, genetic material is deleted in the region of the exchange.

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