BACTERIOLOGICAL REVIEWS, June, 1965 Vol. 29, No. 2
 Printed in U.S.A.

# Chromosome Transfer in Bacterial Conjugation

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## INTRODUCTION

The transfer of chromosomal material from one bacterial cell to another by direct cell-to-cell contact was discovered in 1946 by Lederberg and Tatum (35). This process, called conjugation, has since been shown to occur in many gram-negative bacteria. Most of our knowledge of the mechanisms of conjugation, however, comes from the work of numerous investigators on the organism first used by Lederberg and Tatum, *Escherichia coli* strain K-12. Unless otherwise stated, the data discussed below have all been obtained with this strain and its derivatives.

The first real clue to the mechanism of conjugation was provided by the discovery that chromosome transfer requires the presence in the donor cell of an autonomous, transmissible genetic element (19, 34). This element was called F, for fertility. Since then, a number of other genetic elements have been discovered in bacteria which, like F, promote chromosome transfer. These elements include the several different resistance transfer factors (RTF) which carry a number of genes controlling resistance to antibacterial agents (2, 53), and certain colicinogeny factors, such as Col E and Col I (50), and Col V (31). The latter factors confer on the host cell the property of synthesizing colicines (toxins which are highly specific for strains of *E. coli* other than the colicinogenic host).

Following the terminology proposed by Clark and Adelberg (11), we will refer to all genetic elements which promote chromosome transfer as sex factors, regardless of whether or not they determine other functions, such as resistance to drugs or the formation of colicines. The present paper is an attempt to bring together the information now available concerning the replication and transfer of sex factors and of the chromosome; a general model relating all of the data will be presented. For detailed discussions of many of these observations, the reader is referred to a number of books and reviews (11, 17, 21, 30).

## MATING TYPES IN *E. COLI*

The discovery of F made possible the recognition of two mating types in *E. coli*: $F^+$, harboring F and behaving as genetic donors, or males; and $F^-$, lacking F and behaving as genetic recipients, or females. When a population of $F^+$ cells is mixed with an excess of $F^-$ cells, only about 1 in $10^4$ donor cells transfers chromosomal deoxyribonucleic acid (DNA) to recipients. This transfer is due to the presence in $F^+$ populations of rare mutant types called Hfr, for high frequency of recombination. Upon isolation, such mutants produce populations in which 1% or more of the cells are active donors (10, 18). After this discovery, Jacob and Wollman (27) described a technique for isolating Hfr mutants from $F^+$ populations at will. Studies on Hfr × $F^-$ crosses led, by 1958, to the following picture of chromosome transfer (29):

(i) The $F^+$ chromosome is a closed (circular) structure; the $F^+$ cell contains several identical chromosomes, plus an undetermined number of F particles. (Although the *E. coli* cell typically contains four chromosomes, these segregate at cell division in such a manner that all of the chromosomes in any given cell result from two

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successive replications of a single chromosome. *E. coli* thus behaves as a haploid organism.)

(ii) The conjugation of an $F^+$ cell with an $F^-$ cell leads to the transfer of one or more F particles with an efficiency approaching 100% but only rarely to the transfer of chromosomal DNA.

(iii) As a rare event, an F particle may become attached to the chromosome at one of many possible sites; the cell in which this occurs is called an Hfr mutant. Such attachment has two apparent consequences: F ceases to replicate autonomously; in the clone arising from the Hfr mutant, free F can no longer be detected; and the chromosome breaks at the site of F attachment and is transferred to the recipient in an oriented manner characteristic of the particular Hfr strain. The leading end of the broken chromosome is called Origin; F, the genetic determinant of maleness, is transferred as the last chromosomal marker. The entire process requires approximately 100 min at 37 C.

(iv) In addition to attaching to the chromosome and thus mobilizing it for transfer, F determines at least two other properties required for maleness: the formation of receptor sites on the cell surface to which $F^-$ cells can attach, and the ability to form the type of chemical union necessary for DNA transfer to take place.

To explore the possible mechanisms by which the attachment of F leads to chromosome transfer, it will first be necessary to consider what is known about the nature and replication of each of these elements.

## NATURE AND REPLICATION OF THE BACTERIAL CHROMOSOME

It is not intended to review here all of the information available on the bacterial chromosome, but only that part which is relevant to the question of the transfer mechanism. For a broader coverage, the reader is directed to a number of recent reviews and articles (6, 7, 20, 32).

The first relevant fact is the circularity of the chromosome. In 1956, Jacob and Wollman (28) discovered the closed nature (circularity) of the genetic map of $F^+$ *E. coli*; Hfr cells, however, were believed to have an open (linear) map. Furthermore, it was often pointed out that a circular map does not necessarily imply a circular physical structure. In 1960, however, it was shown by Taylor and Adelberg that Hfr cells which are not acting as donors also possess a circular linkage group (52), and in 1963 Cairns settled the question once and for all by demonstrating that the DNA of *E. coli* can be extracted in the form of a circular molecule approximately one millimeter in length (6, 7). This structure corresponds to the hypothetical chromosome which had been reconstructed from recombination data and from studies of the genetic effects of $P^{32}$ incorporation and decay (29).

By the use of radioautographic techniques, Cairns (6) also showed that the chromosome replicates in the manner illustrated in Fig. 1. The essential features of this process are the following. (i) Replication seems to proceed from only one point on the chromosome at any given moment. If there is more than one potential starting place, the activation of one apparently causes the suppression of the others. A single starting-point for replication has been established independently by Nagata (36) and by Bonhoeffer and Gierer (4). (ii) Although the two strands of DNA have opposite polarity, replication in vivo appears to proceed along both of them in the same direction. (iii) The point at which replication begins [called the "replicator" by Jacob and Brenner (25)] can be inferred to provide at least two functions essential to the replication process. It could allow the double helix to open, providing single strands to act as templates for the DNA replicating enzyme, and it could also function as a swivel. The need for a swivel is a consequence of the circularity of the chromosome; the unduplicated region must rotate in order to unwind, in a sense opposite to the rotation of one of the two arms.

The mechanism by which the replicator acts as a swivel is not known, but it has been suggested by Cairns that the swivel might consist of a single-strand break (6). In that case, the replication process would create a new double helix in which both strands have free ends, since the ends of the newly forming single strands are necessarily free until completion of the replication process and ring-closure. The importance of this consideration for any model of chromosome transfer will become apparent in the following sections.

## NATURE AND REPLICATION OF F

The sex factor ($F_1$) found in wild-type *E. coli* strain K-12 has been shown to be a DNA element approximately 2% of the chromosome in length (15, 48). This corresponds to approximately $10^5$ nucleotide pairs. Recently, Rownd (*personal communication*) compared the profiles of the DNA from $F^-$ and $F^+$ cells by equilibrium density-gradient centrifugation, and found a shoulder in the $F^+$ profile corresponding to a DNA containing 44% guanine-cytosine (GC). The chromosomal DNA, on the other hand, contains 50% GC. Of particular interest is Rownd's estimate that the 44% GC material is equivalent to only about 0.1% of the chromosomal DNA, whereas the measurements of the total sex factor size indicate it to be equivalent to 2% of the chromo-

somal DNA. These differences can be reconciled, however, by assuming that the sex factor consists of a small piece of 44% GC DNA attached to a much larger piece of 50% GC DNA. As will be shown later, such an assumption would account for many of the observations concerning F integration and chromosome transfer by Hfr cells.

Rownd's observations have been confirmed and extended by Falkow and Citarella (16). These authors measured the homology between F DNA and chromosomal DNA by isolating the DNA of F-genotes after their transfer to bacteria with a widely different GC content, and measuring its adsorption to various types of bacterial DNA suspended in agar-gel columns. Their results show clearly that wild-type F is 1.9% of

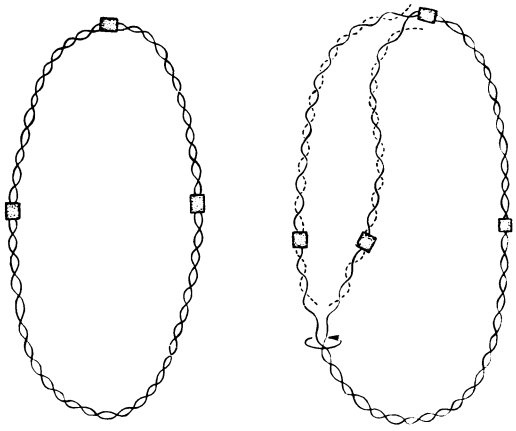

FIG. 1. *Replication of the bacterial chromosome, according to Cairns (1963). The squares represent hypothetical replicators. Newly synthesized DNA strands are shown as broken lines, and replication is shown as proceeding counter-clockwise from the replicator at the top of the figure, which also serves as a swivel for the unwinding of the two old strands.*

the bacterial chromosome in size, and consists of two regions: one region, making up 10% of F, contains DNA of 44% GC content; the other region, making up the bulk of F, contains DNA of 50% GC content. Half of the latter region is hybridizable with chromosomal DNA.

Although the F genome has been neither genetically mapped nor physically isolated, there are reasons to believe that it will ultimately prove to be circular. The reasons for so believing are as follows. (i) F shares with the chromosome and with bacteriophage genetic material the ability to replicate as an independent unit. It is thus, by definition, a "replicon" (25). Replicons which are now known to be circular structures include the bacterial chromosome (6), as well as bacteriophage λ (45). F further shares with λ the ability to integrate with the chromosome and to incorporate segments of chromosome upon reverting to the autonomous state. All of these similarities suggest a similar physical structure. (ii) A sex factor which has incorporated a chromosomal segment (an F-genote) is subject to genetic mapping. (The nature and formation of F-genotes will be discussed in Formation of F-Genotes.) Preliminary experiments using phage transduction have given results which are compatible with a circular structure for the F-genote $F_{14}$ (Pittard, *unpublished data*). (iii) Finally, an analysis of crossing-over between $F_{14}$ and the chromosome has also provided suggestive evidence for the circularity of $F_{14}$ (39).

Little is known about the replication of F, but some experiments of Jacob, Brenner, and Cuzin (26) suggest that it is essentially similar to the replication of the chromosome. Mutations were found to occur on the sex factor which permit it to replicate at low temperature but not at high temperature. Such experiments, together with observations on the rate of F multiplication in newly infected cells (14), suggest that the replication of F is independently controlled, presumably by the action of a gene product on an F replicator. In the absence of evidence to the contrary, then, we shall assume that replication of F, like that of the chromosome, proceeds in the manner shown in Fig. 1.

The number of F replicons in an $F^+$ cell has never been directly determined. However, estimates have been made in the case of certain F-genotes, such as F-*lac* and $F_{14}$, by measuring the levels of enzymes controlled by genes which have been incorporated into the F-genote (26, 41), or by measuring the relative probabilities of a mutation occurring on the F-genote or on the chromosome (43). These estimates have varied from one F-genote per chromosome (26, 41) to three per chromosome (43). To account for the regular distribution of F and chromosomal replicons at cell division, it has been postulated that these elements attach to the newly forming cross-wall at the time of their replication, in such a manner that the replication products are segregated (26, 46).

## INTEGRATION OF SEX FACTOR AND CHROMOSOME

The autonomous replicons which are known to be capable of attachment to the chromosome include F and λ. Campbell (9) presented a model for the attachment of λ to the chromosome based on pairing of the two circular structures followed by breakage and reunion (crossing-over). Although direct proof of the model is still lacking, it does account for the different marker order

found when λ is mapped by vegetative recombination and by prophage recombination (8).

Campbell's model stems primarily from observations on the structure of λdg, the defective prophage in which the *gal* loci of the chromosome have replaced a segment of the phage genome. Although less is known about the structure of F-genotes, the similarities between them and such transducing phages as λdg suggest a common mechanism for their origin. We will assume this to be so, and will further assume that Campbell's model for λdg formation is the correct one. These assumptions constitute two of the postulates on which our general model for chromosome transfer will be based.

In Campbell's model, the crossing-over event responsible for λ integration requires the presence in the chromosome of a region of homology

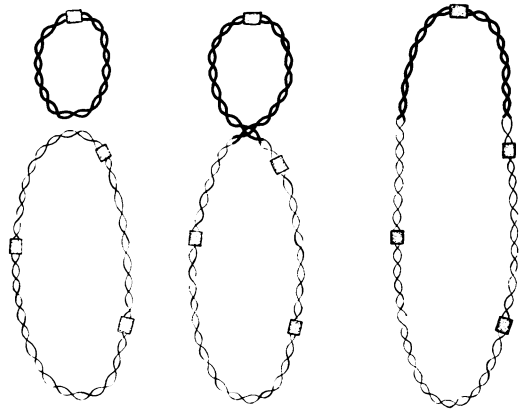

FIG. 2. *Integration of sex factor (heavy lines) and chromosome (light lines) by breakage and reunion.*

with a site in the phage genome, i.e., a region of identical base-pair sequence. *E. coli* K-12 has one attachment site for λ, close to the *gal* locus. In the case of the sex factor, $F_1$, there are 10 to 12 known chromosomal sites at which integration can take place; these will be considered to be regions of homology with one or several regions of the F genome. According to our model, which is essentially the model proposed by Stern (51), pairing of $F_1$ and chromosome occurs as a rare event at one or another of these regions; once pairing has occurred, a crossover within the region integrates the two replicons. For example, one such pairing region is at the histidine operon. If we designate certain *his* mutational sites within the operon as A and B, integration of F can occur by a crossover between markers A and B or to the left of both (Matney, *personal communication*). Similarly, a pairing region exists at the *lac* operon, but the crossover which integrates the sex factor can take place either at one end of the operon or between mutational sites in the Z cistron (12).

Figure 2 illustrates the integration of F and chromosome according to these assumptions. The chromosome of the $F^+$ cells is pictured as having several replicators; the chromosome of the Hfr cell is shown with one additional replicator, that of the integrated sex factor. The existence of several chromosomal replicators is inferred from the experiments of Nagata (36), in which synchrony of marker replication could be achieved

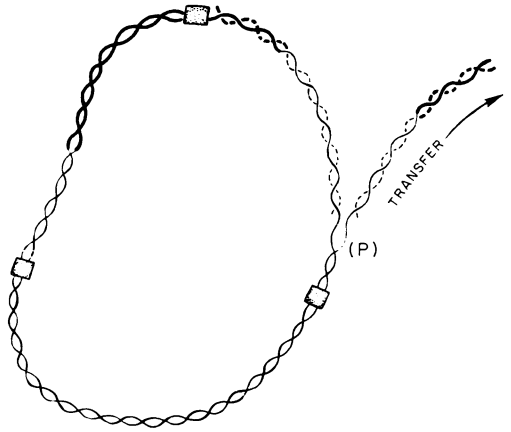


FIG. 3. *Jacob-Brenner model of chromosome transfer. Replication has begun at the F replicator at the top of the figure, and has proceeded in the clockwise direction. One of the daughter double helices is transferred in the direction shown by the arrow. The point at which DNA synthesis is taking place would be the location of the DNA polymerase (P), which is pictured as being fixed to a point on the bacterial membrane. The entire chromosome then moves past that point, in the counter-clockwise direction.*

for a population of Hfr cells but not for a population of $F^-$ cells. This was interpreted to mean that chromosomal replication can start at only one site in Hfr cells (at the F replicator) but at any one of several alternative sites in $F^-$ or $F^+$ cells. Positive evidence is lacking, however, and it is possible that only one chromosomal replicator exists.

Regardless of whether the $F^+$ chromosome has one or several replicators, Nagata's experiments suggest that chromosomal replicators cease to function in Hfr bacteria, replication beginning only at the replicator of the integrated sex factor. Once replication has begun there, it proceeds around the entire F-chromosome structure; in

other words, F and chromosome now behave as a single replicon. [It should be noted that in the model of Jacob and Brenner (25) discussed below, F and chromosome are also proposed to constitute a single replicon in the Hfr cell. In their model, however, replication is postulated to start at a chromosomal replicator rather than at the F replicator. This postulate is based on the observation that acridine orange inhibits the replication of autonomous F at a concentration to which replication of the integrated replicon is resistant.]

The chromosomal site at which F integration takes place is known from studies of the transfer of chromosome by Hfr strains during conjugation. This process will now be examined in more detail.

## CHROMOSOME TRANSFER BY HFR STRAINS

Two questions can now be asked about the transfer process: (i) what causes the circular chromosome of the Hfr cell to break at the time of conjugation, and (ii) what causes the chromosome to move from the male to the female cell?

The first question can be answered by defining chromosome "breakage" as the creation of a free end. As discussed earlier, this is just what happens when the circular chromosome replicates. To explain the fact that during conjugation the Hfr chromosome breaks at the inserted sex factor, Jacob and Brenner (25) proposed the following model: chromosome replication in the nonconjugating Hfr cell begins at a chromosomal replicator; the event of conjugation triggers the start of a new round or replication beginning at the F replicator, such that the Origin (the leading point in transfer) is duplicated first. One daughter replica penetrates the female cell, moving at the expense of the free energy liberated by the polymerization of nucleoside triphosphates. Transfer is thus pictured as being directly geared to replication (Fig. 3).

The Jacob-Brenner model, however, does not take into account Nagata's evidence that replication of the Hfr chromosome begins at the F replicator even in nonconjugating cells, and that proceeds in the opposite direction; i.e., the Origin is the last point to be duplicated, rather than the first. With this in mind, Bouck and Adelberg (5) proposed a somewhat different model. According to their model, replication begins at F and proceeds in the sense shown by Nagata. At the moment that a population of Hfr cells is mated, every cell is in a different stage of the replication cycle. As each conjugating cell completes its replication, one of the replicas fails to undergo ring-closure. Instead, the free end created becomes the Origin, and transfer commences (Fig. 4). This model says that DNA synthesis in a culture of Hfr cells is necessary for the *initiation* of chromosome transfer, but may not be necessary for the transfer process itself.

The two models lead to a number of different predictions. The Jacob-Brenner model, for example, says that if DNA synthesis is halted after transfer has been initiated, transfer should stop. The Bouck-Adelberg model, on the other hand, leaves open the possibility that transfer may continue. To test these predictions, Bouck and Adelberg allowed Hfr cells to initiate transfer for different lengths of time and then treated them with phenethyl alcohol (PEA), which specifically inhibits DNA synthesis (3). After allowing an additional period of time for transfer to take place, the mating couples were sheared apart and

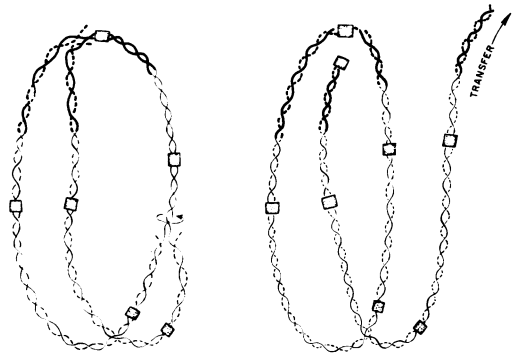


FIG. 4. *Bouck-Adelberg model of chromosome transfer. Left: replication has begun at the F replicator at the top of the figure, and is proceeding in the counter-clockwise direction. Right: replication has been completed, but ring-closure of one daughter chromosome has been prevented by the initiation of its transfer to an F⁻ recipient.*

plated to assay for recombinants. Their results clearly showed that some transfer does take place in the absence of net DNA synthesis; Jacob, Brenner, and Cuzin (26) demonstrated, however, that some DNA *turnover* takes place after PEA addition, and applied their model to Bouck and Adelberg's results by attributing the observed transfer to residual DNA synthesis. It is not yet clear which interpretation is correct.

Another prediction in which the two models differ has to do with the speed of chromosome transfer. The Jacob-Brenner model says that the speed of transfer is directly related to the rate of DNA synthesis, whereas the alternate hypothesis makes no such prediction. It has now been shown that the time of entry of markers, which measures the speed of transfer, does not change when the rate of DNA synthesis in the cell population is

drastically lowered by thymine starvation (42) or by PEA treatment (Bouck and Adelberg, *unpublished data*). It is possible, however, that, for any given cell, DNA synthesis continues at its maximal rate until it is stopped by the treatment in question. The decline in rate measured for the whole population would then reflect the accumulation of cells in which DNA synthesis had stopped, and the observed residual transfer could have been effected by cells in which the rate of DNA synthesis was maximal.

There are other differences in the predictions made by the two models, and these are currently being tested in several laboratories. The two models agree, however, in one important prediction: they both say that, of the DNA which is transferred early, one strand is made during conjugation. Thus, if the males are grown in heavy medium ($C^{13}N^{15}$) and mated in light medium ($C^{12}N^{14}$), the early-transferred DNA should contain one light and one heavy strand. The Jacob-Brenner model says that all of the transferred DNA should be hybrid; the Bouck-Adelberg model says that the material transferred earliest will be hybrid, whereas the material transferred later will be all heavy. The late material may not be found in detectable amounts, however, since chromosome breakage occurs during transfer to such an extent that the average cell transfers only 16% of its DNA during an uninterrupted mating (11, 48).

Jacob, Brenner, and Cuzin (26) verified the general prediction by carrying out the experiment described above, isolating the DNA from the zygotes after destruction of the male cells. The amount of heavy-heavy DNA which they found, in addition to the predicted hybrid DNA, could have come either from contaminating Hfr DNA or from DNA transferred according to the Bouck-Adelberg model. Very little of the latter would be expected, since conjugation was interrupted at 25 min when transfer is only 30% complete.

At this moment, then, it can be said with some confidence that transfer of the Hfr chromosome is initiated as a result of replication of the Origin, either as the end of a cycle of replication or as the start of a new one. Whether the transfer itself requires DNA synthesis as a source of energy is still open to question. A strong possibility exists that both models are correct: conjugation may trigger F-replication with consequent transfer, but this process may have to wait until the current reverse cycle of chromosomal replication has been completed.

A recent report by Roeser and Konetzka (45*a*) is not incompatible with this view. Their data show that PEA permits cells to complete their current cycle of replication but prevents initiation of the next cycle. If the PEA is removed after completion of the cycle, DNA replication immediately recommences; the addition of PEA 20 min later permits completion of the second cycle over a further 100-min period. This system has permitted a more refined test of the correlation between DNA synthesis and transfer. Donor cells which had begun a cycle of replication and had then been treated with PEA were mated with $F^-$ cells. No transfer was observed, even though most of the cells went on to complete the current cycle of replication. Along with appropriate controls, this observation indicates that transfer requires the initiation of a cycle of DNA synthesis during mating, not just the completion of a cycle.

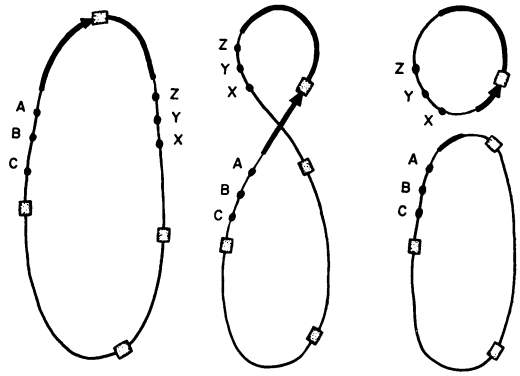


FIG. 5. *Formation of an F-genote by imperfect relooping of the F-chromosome integrated replicon. F DNA is indicated by the heavy lines; chromosomal DNA by the light lines. A segment of F DNA remains in the shortened chromosome.*

## FORMATION OF F-GENOTES

In Integration of Sex Factor and Chromosome, Campbell's model was presented for the integration of F and chromosome. The reversal of the process pictured in Fig. 2 would lead to a change from the Hfr state back to the $F^+$ state. On rare occasions, however, the Hfr chromosome might "reloop" imperfectly; breakage and reunion in such a structure would produce a sex factor carrying a piece of chromosomal DNA, as well as a chromosome in which a number of loci have been deleted and replaced by F DNA (Fig. 5).

This model, first proposed by Campbell to explain the formation of λdg, is compatible with all of the known facts about F-genotes. Strains carrying F-genotes (F′ strains) have been selected in the following manner (24). A large population of Hfr cells is mated for a limited period of time

(e.g., 30 min) with a suitable $F^-$, and is then destroyed by phage. If we consider the Hfr to carry markers $A^+$ through $Z^+$ in which $A^+$ is transferred first and $Z^+$ last, no Hfr cell will transfer marker $Z^+$ in the 30 min of mating. If, however, there are any "F′ mutants" in the population in which marker $Z^+$ has become integrated into the sex factor, the element $F\text{-}Z^+$ will be transferred within the first few minutes of mating. If the female strain used is $Z^-$, selection for $Z^+$ recombinants at 30 min will permit the isolation of $F\text{-}Z^+/Z^-$ heterozygotes. This is illustrated in Fig. 6, in which markers X, Y, and Z are shown to have been transferred as part of an F-genote.

Campbell's model makes two other interesting predictions. One is that the cell in which the F-genote is formed should now contain a chromosome bearing a piece of sex factor DNA. Although there is no way of selecting for the cell in which the original event took place, a clone arising from such a cell was accidentally discovered (1). When cells of this clone were treated with acridine orange to remove the F-genote (22), an $F^-$ strain was obtained which exhibits a remarkable property. When this strain is infected with wild-type F, it becomes a high-frequency donor of chromosomal markers. The order of marker transfer shows that breakage always occurs at the site of original F attachment. This is just what is predicted if Campbell's model is correct and the chromosome contains a piece of sex factor DNA at the former attachment site. The new F put into the cell should pair with the homologous F DNA in the chromosome, bringing about breakage and transfer of the chromosome in the manner described in the following section. The incorporated fragment of F DNA has been termed an *sfa* locus (for sex factor affinity). Two such *sfa* loci have been described (1, 44).

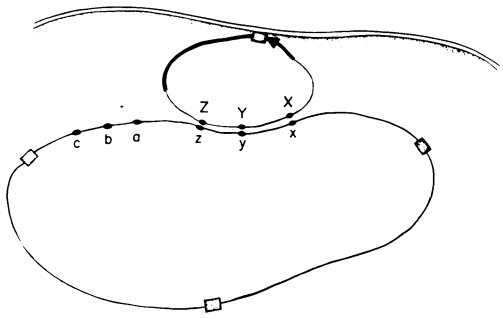


FIG. 6. *F-genote shown forming in Fig. 5 is now shown in a recipient cell to which it has been transferred. Pairing has occurred between homologous regions on the F-genote and on the chromosome. The F-genote is shown attached to the bacterial membrane by its replicator.*

The other prediction made by Campbell's model is that early markers (e.g., $A^+$) should be incorporated into F as often as late markers. Recently, a method for detecting such events was devised by Curtiss (12). He selected for the early transfer of both $A^+$ and $Z^+$ to a recipient, which was $A^-Z^-$; an F-genote was thus selected carrying both "early" and "late" Hfr markers, and the diploid strain formed had the genotype $F\text{-}A^+Z^+/A^-Z^-$. In this case the markers "A" and "Z" were two different loci concerned with proline biosynthesis.

## TRANSFER OF F-GENOTES BY F′ STRAINS

When an F′ strain such as that pictured in Fig. 6 is mating with a suitably marked $F^-$ strain, the F-genote is transferred at a frequency approaching 100%. Breakage of the F-genote occurs at a specific site (inferred to be the F-replicator) and the F-genote markers are sequentially transferred in the order *X*, *Y*, *Z*, *F*. This was first shown by Hirota and Sneath (23) for a sex factor carrying the markers *ade*, *T6-r*, and *lac*; more recently, Pittard and Adelberg (39) carried out a kinetic analysis of the transfer of an exceptionally long F-genote, $F_{14}$. The DNA of $F_{14}$ represents approximately 10% of the chromosome, and requires 9 min to be transferred. The earliest marker to be transferred by $F_{14}$ is *met-1*, and the last marker is *ile-1*; the time required to transfer the *met-ile* segment is the same, whether it is part of the sex factor replicon or part of the chromosome. The fact that both F-genote and Hfr chromosome are transferred at the same speed speaks strongly for a common mechanism.

## TRANSFER OF CHROMOSOME BY F′ STRAINS

With one important exception, to be discussed later, all F′ strains transfer chromosome at moderate frequencies. In each case, the chromosome appears to break at the site characteristic of the Hfr in which the F-genote arose. For example, Hfr strain AB313 transfers *xyl* as an early marker with a frequency of about 40%. When $F_{14}$, which arose in an AB313 cell, is put into an $F^-$ to make an F′ strain, $F_{14}$ itself is transferred at a frequency of 50%, whereas *xyl* is still transferred early but at a frequency of only 5%.

Pittard and Adelberg (38) have studied the kinetics of transfer of $F_{14}$ markers and chromosomal markers by F′ cells. Zygotes were selected which had received the chromosomal marker $xyl^+$, and these were analyzed for the presence of $F_{14}$ markers $met^+$, $arg^+$, and $ilv^+$. Large excesses of $F^-$ cells were used in these crosses, to ensure

that each zygote arose from a mating involving a single F′ cell. The percentage of *xyl*$^+$ recombinants containing $F_{14}$ markers did not vary with the time at which the matings were interrupted, indicating that the $F_{14}$ markers preceded the chromosomal marker in transfer. However, the percentage did vary with the $F_{14}$ marker: *met*$^+$, the earliest $F_{14}$ marker to be transferred, was recovered at 70%; *arg*$^+$, the next marker to be transferred, was recovered at 30%; and *ilv*$^+$, the last marker to be transferred, was recovered at 20%. These findings were interpreted to mean that chromosome transfer causes the breakage of $F_{14}$ in transit.

The true nature of this breakage was revealed by the work of Scaife and Gross (47). Their studies on chromosome transfer by F′ strains carrying F-*lac* showed that the breakage is, in reality, a crossover between F-genote and

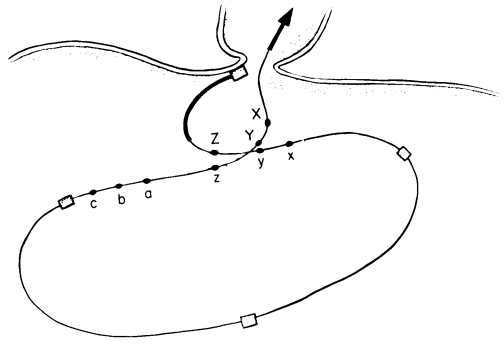


FIG. 7. *F′ cell is shown transferring its F-genote to a conjugated F$^-$ cell. A crossover has taken place between the F-genote and the chromosome.*

chromosome. Guided by this interpretation, Pittard and Adelberg (39) carried out exhaustive analyses of crossing-over events in both the F′ donor and in the zygote. Their conclusions can be summarized as follows: F′ strains transfer their F-genotes with close to 100% efficiency. Chromosome transfer occurs in such strains solely as a consequence of crossing over with the F-genote. In the donor cell, the probability of a crossover is constant per unit length of F-genote; in the zygote, the probability of a crossover is also constant per unit length of the transferred element, with the exception that in the very small region adjacent to the leading end the crossover frequency is elevated about 40-fold. [The elevated crossover frequency in the proximal region of the F-genote in the zygote, but not in the donor cell, is consistent with the model of a circular F-genote in the donor and a linear (broken) F-genote in the zygote.] A given F′ male cell can transfer both chromosome (integrated with the F-genote) and a second entire copy of the F-genote to the same recipient.

The transfer of chromosome by F′ strains thus depends on crossing over in the region of homology between the chromosome and F-genote (Fig. 7). If no region of homology exists, no chromosome transfer occurs. For example, strain AB1206, which harbors $F_{14}$, has a chromosomal deletion corresponding to the entire F-genote (41). Chromosome transfer by AB1206 is about $10^{-3}$ that of other $F_{14}$ strains, and is barely detectable.

Examination of Fig. 7 leads to the prediction that, if the crossing-over model is correct, a given chromosomal marker should be transferred later in an F′ strain than in the parental Hfr strain by the number of minutes required to transfer the F-genote. This prediction has been verified (40):

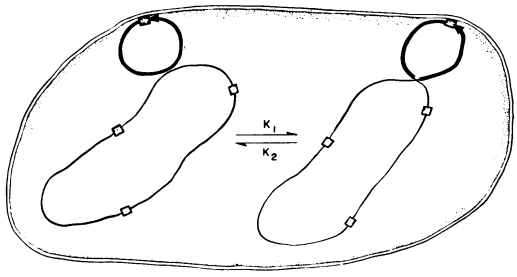


FIG. 8. *Dynamic equilibrium between the integrated and nonintegrated states of the sex factor and chromosome is shown schematically. $K_1$ and $K_2$ are rate constants for the events of integration and separation. In the F′ system, $K_1$ and $K_2$ are approximately 0.1 and 0.9 per cell per generation, respectively. In the F$^+$ ⇄ Hfr system, $K_1$ and $K_2$ are both approximately $1 \times 10^{-5}$ per cell per generation.*

the *xyl* and *mal* markers are transferred at 18 and 25 min, respectively, by Hfr strain AB313, but at 28 and 35 min, respectively, by the derived F′ strain, AB1516. The F-genote in AB1516 requires approximately 9 min to be transferred.

## GENERAL MODEL FOR CHROMOSOME TRANSFER

In Chromosome Transfer by Hfr Strains we summarized the evidence for believing that, in Hfr cells, chromosome breakage and transfer are consequences of replication beginning (or ending) at the replicator of the integrated sex factor. What has been inferred to be true for the F-chromosome replicon of the Hfr cell is presumed to be true also for the F-genote of the F′ cell: replication of F leads to the transfer both of F and of the chromosomal DNA with which it has become integrated. In F′ cells, as we have shown above, the F-genote is the primary transferable

element; the chromosome itself is transferred only by virtue of a crossover with it.

The crossing over which takes place in F′ cells appears to be a very frequent and highly reversible process (Fig. 8). Such crossing over does not, as a rule, produce clones of cells in which chromosome and F-genote are stably integrated. Instead, each cell in an F′ population grows into a clone in which, at any given moment, some of the cells have an autonomous F-genote and some have an integrated F-genote. As a rare event, however, one or the other of the two states can become stabilized. In AB1206, for example, the autonomous state has become stabilized as the result of a chromosomal deletion which prevents pairing and crossing over (see above), whereas Cuzin and Jacob (13) reported a strain in which the integrated state has become stabilized after an unknown mutational event.

The results of the studies on the F′ strains throw new light on the integration of F and chromosome which converts $F^+$ cells to Hfr. It will be recalled (Nature and Replication of F) that F contains two types of DNA: one with a GC content of 44%, and the other with a GC content similar to that of the *E. coli* chromosome (50%). The latter material provides homology with certain regions of the chromosome; a crossover within one of these homology regions would lead to integration of F at a characteristic site.

$F_1$, the sex factor found in the wild-type strain K-12 of *E. coli*, can thus be considered to be a unique F-genote in which the chromosomal DNA corresponds to a number of small chromosomal regions instead of to one large one. The manner in which such an F-genote might have arisen will be discussed in the following section. The crossover which produces an Hfr strain from an $F^+$ strain carrying $F_1$ and the crossover which leads to chromosome transfer by an F′ strain may be considered to be completely analogous, and to differ only in their apparent reversibility: in the $F^+$ to Hfr transition the integration appears to be highly stable, whereas in the F′ cell the integration appears to be highly reversible.

Actually, as anyone who has worked with Hfr strains will appreciate, the integration is reversible in both cases. The *rates* of the transitions in both directions are very different, however. In Fig. 8, some approximate rate constants have been assigned to illustrate this difference. In the F′ strain, the probability of a crossover leading to integration during a single division cycle is on the order of $1 \times 10^{-1}$, and in the other direction is on the order of $9 \times 10^{-1}$, based on the proportions of cells which transfer integrated and nonintegrated F-genotes when F′ populations are mated. The two states are thus visualized as being in a state of rapid equilibrium.

In the $F^+ \leftrightarrow$ Hfr transition, however, the rate in both directions is on the order of $10^{-5}$ per cell division cycle. Thus, if one isolates an $F^+$ strain, the mutation rate to Hfr is about $10^{-5}$ per generation and the proportion of Hfr cells in the culture increases very slowly. Conversely, if one isolates an Hfr strain, the mutation rate to $F^+$ may also be about $10^{-5}$ per generation (although this varies from one Hfr to another), and the proportion of $F^+$ cells in the culture increases very slowly. In both cases mutational equilibrium would be reached after about $10^5$ generations, but this is not likely to be observed. For one thing, differences in growth rate affect the proportion of the two types far more than does mutation pressure, and, for another, the constant reisolation of strains from single cells that is practiced by all bacterial geneticists usually prevents equilibrium from being approached. Neither factor, however, can interfere with the rapid attainment of equilibrium which occurs in F′ cells.

The most likely explanation for the different rates at which $F^+$ and Hfr cells, on the one hand, and the two types of F′ cells, on the other hand, approach equilibrium would seem to be the different amounts of chromosomal DNA which the F-genote contains in the two instances. The larger the region of homology, the greater the frequency at which pairing and crossing over will occur; hence the rapidity with which equilibrium is attained in F′ cells.

## DISCUSSION

Many features of the model described above have been either explicit or implicit in the recent publications of Jacob, Brenner, and Cuzin (26) and of Scaife and Gross (47), as well as in our own. The purpose of this article has been to bring all of the current observations and speculations together in one review.

It is apparent that practically all of the experimental work on sex factor function has been done with $F_1$, the sex factor found in *E. coli* strain K-12. The other known sex factors, such as RTF [Watanabe (53)] and *col* factors (31, 37) may be presumed to promote chromosome transfer by the same general mechanism, but direct evidence for this belief is lacking. What is known about these factors is compatible, however, with the view that they are analogous to F-genotes. A number of *col* factors have been shown by Silver and Ozeki to consist of DNA and to be of the same order of size as F (49), and one of the RTF agents has been shown to be capable of attachment to the chromosome (53).

According to the general model, then, a sex factor must contain a unique segment of DNA which includes the genetic determinants for the mating reaction and for the transfer of the sex factor itself; in addition, it must contain one or more segments of DNA which are homologous with regions of the host chromosome. Either in the unique segment or in the homology segments there must be a replicator, capable of responding to an initiator produced in the cell.

The unique segment (e.g., the segment of $F_1$ which contains 44% GC) is foreign to the host cell, and its origin is completely obscure. It could have been derived from a bacterial virus, but other sources of foreign DNA are easily imagined. The homology segments, however, seem likely to have been picked up as the result of chance integrations of sex factor and chromosome at novel sites, followed by an imperfect reversion to the autonomous state as pictured in Formation of F-Genotes for the origin of F-genotes. In the case of $F_1$, such an event would have to have occurred 10 or 12 times at as many different sites during the evolution of the F-K-12 system, to explain the types of Hfr strains to which this system now gives rise. It is not known how many homology sites there are in the case of the RTF and colicinogeny sex factors, although the presence in the former of several unrelated resistance loci suggests that chromosomal fragment "pickup" has occurred more than once. The presence on one sex factor of several different chromosomal fragments could be the result of successive pick-up events, recombination between sex factors, or both. The biggest question remaining to be answered is that of the mechanism by which sex factors bring about their own transfer. A clue to the problem may lie in the recently discovered attachment of bacterial DNA to mesosomes, which are invaginations of the cell membrane (33, 46). It has been suggested (26) that the sex factor is also attached to the membrane, and causes the formation of a receptor site for conjugation on the cell surface immediately opposite the attachment site. Conjugation then triggers, in an unknown manner, the transfer of the sex factor as well as of any other donor DNA which is integrated with it.

The transfer process is still a mystery. Jacob and Brenner (25) suggested that it is driven by F replication. It should be pointed out, however, that the transfer of F is not unlike the injection of phage DNA into a host cell, and it is possible that both phage injection and F transfer take place by the same mechanism. If so, replication need not be involved as the source of energy for transfer, although the experiments described in Chromosome Transfer by Hfr Strains show that it is indeed necessary for the initiation of transfer, presumably because it provides a means of breaking the "circular" chromosome.

The general model which has been proposed seems to be compatible with the observations made by Clark (10*a*) on a double male strain of *E. coli* K-12. This strain, prepared by crossing two different Hfr strains, has a sex factor integrated at each of two different sites on the bacterial chromosome. When the double male is mated with an $F^-$ strain, its chromosome appears to break at both sites; two chromosomal segments are thus generated, and each male cell in the population transfers one or the other segment (but not both simultaneously). These observations can be explained by assuming that F-replication and transfer start at one or the other of the sex factors, but that the chromosome is broken by the initiation of replication at the second sex factor. A given male cell transfers whichever segment of the chromosome happens to begin replication first, as a consequence of conjugation.

The current hypothesis of chromosomal transfer, in which the leading point is a site within the DNA of the integrated sex factor, seems at first glance to contradict the often-repeated statement that the sex factor is linked to the last marker to be transferred. This statement is based on the observation that only those recombinants which receive the entire donor chromosome are males, an observation which is fully compatible with the hypothesis of breakage within the sex factor. Indeed, evidence for a part of the sex factor being present at the origin, or leading, end of the chromosome was presented several years ago (1, 54). These earlier findings lend further support to the current hypothesis of chromosome transfer.

## SUMMARY

Bacteria are host to a number of autonomous genetic elements, or plasmids, with the following properties: (i) they are composed of DNA; (ii) they determine one or more phenotypic properties of the host; (iii) they replicate in the host cell autonomously; (iv) they promote conjugation with other bacteria; and (v) conjugation leads to their own transfer.

These elements, which we will call *autotransferable*, include the Resistance Transfer Factors (RTF), colicinogeny factors (Col), and the F factor of *E. coli* strain K-12. Some autotransferable elements appear to have incorporated segments of bacterial chromosome which are capable of pairing with homologous regions of the host genetic material. Crossing over in paired regions brings about the integration of the host chromo-

some with the autotransferable element, so that when conjugation occurs, the entire integrated structure is transferred. Autotransferable elements which promote chromosome transfer in this manner are called *sex factors*.

Studies on $F^+$, F′, and Hfr strains of *E. coli* K-12 indicate that replication of F is an essential step in the transfer process. Two models have been proposed: in one, replication is essential only for the initiation of transfer; in the other, the replication process provides the energy for the transfer act itself. The experimental evidence obtained to date does not rule out either model; it is possible that both are correct. In both models it is visualized that autotransferable plasmids attach to the cell membrane and govern the formation of conjugation receptors on the cell surface directly opposite the attachment sites. Conjugation triggers the transfer of the plasmid, along with any other DNA which has become integrated with it. The bacterial chromosome is transferred in conjugation as a result of its integration with a sex factor.

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