DocuServe

Electronic Delivery Cover Sheet

WARNING CONCERNING COPYRIGHT RESTRICTIONS

The copyright law of the United States (Title 17, United States Code) governs the making of photocopies or other reproductions of copyrighted materials. Under certain conditions specified in the law, libraries and archives are authorized to furnish a photocopy or other reproduction. One of these specified conditions is that the photocopy or reproduction is not to be "used for any purpose other than private study, scholarship, or research". If a user makes a request for, or later uses, a photocopy or reproduction for purposes in excess of "fair use", that user may be liable for copyright infringement. This institution reserves the right to refuse to accept a copying order if, in its judgment, fulfillment of the order would involve violation of copyright law.

Caltech Library Services

ILL

TN #: 707440

ILL #: -15510296

RAPID:CIT

Shipping Option: Odyssey

Patron:

EFTS:

Notice: This material may be protected By copyright law (Title 17 U.S. Code)

Odyssey: docuserve.library.caltech.e

RAPID:CIT
NEW: Caltech Library

Ariel:
Odyssey: docuserve.library.caltech.edu
e-mail:
E-MAIL:
Fax:
Phone:
Maxcost:

Comments:
Notes:
Need by:

Caltech Library Services

Journal Title: Symposia of the Society for Experimental Biology

Volume: 12 **Issue:**
Month/Year: 1958 **Pages:** 75-92

Article Author: JACOB F,

Article Title: Genetic and physical determinations of chromosomal segments in Escherichia coli.

Imprint:
Item #:
Call #: 39072020970772
Location: Libraries Annex: Empire Shared Non-Circulating Collection

Copyright Charges:

Date Received: 12/2/2019 11:53:39 AM

Status:

Date Cancelled:
Reason Cancelled:

Date Sent:
Number of Pages:

University at Buffalo Libraries, NEW: Caltech Library
BUF NYUSBU
716-645-2812 buflend@buffalo.edu

BUF - Lending

GENETIC AND PHYSICAL DETERMINATIONS OF CHROMOSOMAL SEGMENTS IN *ESCHERICHIA COLI*

By FRANÇOIS JACOB and ELIE L. WOLLMAN
Service de Physiologie microbienne, Institut Pasteur, Paris

In *Escherichia coli* K-12, conjugation involves the *oriented transfer* of a chromosomal segment from a donor to a recipient bacterium. This process is oriented in the sense that the different genetic determinants located on the chromosomal segment transferred from the donor penetrate into the recipient in a predetermined order, and according to a rather precise time schedule (Wollman & Jacob, 1955). This characteristic of the mating process makes conjugation in bacteria a suitable material for relating genetic analysis to genetic structures, and for comparing genetic evaluation of these structures with physical measurements.

After summarizing our present knowledge of the process of conjugation in *E. coli* K-12, it is intended, in the first part of this paper, to report the available information on the organization of the genetic material in this organism. The second part will be mainly concerned with the effects of ultra-violet light and of disintegration of radioactive phosphorus on the processes of conjugation and recombination. These last experiments allow, as will be discussed, a comparison between genetic and physical determinations of chromosomal segments in bacterial crosses.

I. THE GENETIC SYSTEM OF *E. COLI* K-12

The process of conjugation

When two strains of *E. coli* K-12, which differ in such properties as synthesis of essential metabolites, fermentation of sugars, resistance or sensitivity to bacteriophages or to drugs, are mixed, genetic recombination may be demonstrated between characters of the parental types. In the recombinants thus formed the characters of the parents are reassorted and certain types of recombinants may be easily scored by plating on suitable selective media (Tatum & Lederberg, 1947; Lederberg, 1947). Sexual differentiation in *E. coli* K-12 has been demonstrated, and genetic recombination involves the transfer of genetic material from *donor* to *recipient* bacteria (Hayes, 1953*a*; Cavalli, Lederberg & Lederberg, 1953). Whereas no essential difference has been recognized between different

strains of recipient (or, F^-) bacteria, two main types of donors may be distinguished. Most of the K-12 strains, including the wild type, are F^+: upon mixing with F^- cells they exhibit a low frequency of recombination (10^{-5} or less of any type of recombinant). Some strains, however, exhibit a high frequency of recombination (from 10^{-1} to 10^{-3}) and are thus called *Hfr* (Cavalli, 1950; Hayes, 1953*b*). Closer analysis shows that only certain characters of an *Hfr* strain are transmitted at high frequency to recombinants, whereas others are transmitted at the same low frequency as is observed in $F^+ \times F^-$ crosses (Hayes, 1953*b*).

The high frequency of recombination that may be observed in $Hfr \times F^-$ crosses allows an analysis of the process of conjugation at the cell level (Wollman, Jacob & Hayes, 1956). One may schematically distinguish several successive steps in this process (Jacob & Wollman, 1955).

(i) The first step consists in the establishment of an *effective contact* between cells of opposite mating types. Under suitable conditions of environment and cell density this step is rapidly completed and practically all possible matings occur within 30 min. after mixing of bacteria of opposite mating types. Electron micrographs demonstrate the existence of a bridge which unites conjugating bacteria (Anderson, Wollman & Jacob, 1957).

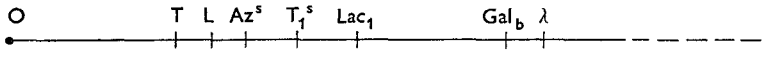
(ii) The second step consists in the oriented *transfer* of a chromosomal segment of the *Hfr* donor into the F^- recipient. The mechanism of transfer has been analysed by interrupting the process of conjugation at different times after its onset, by means of a Waring blendor (Wollman & Jacob, 1955). It was thus found that the genetic characters linearly arranged on the *Hfr* chromosome segment penetrate into the recipient in a predetermined order and always the same extremity, O (for origin), first. This process is slow enough to be interrupted at various times by mechanical treatment. Interruption of the mating process does not prevent any genetic character which has already penetrated the recipient from being later integrated into a recombinant. Even when the process is not artificially interrupted, spontaneous breaks do occur during transfer of the *Hfr* chromosome, with the result that the length of the segment transferred varies from one mating pair to the other. As a result of transfer a partial zygote or *merozygote* is formed, which comprises the whole F^- recipient and a chromosomal segment of *Hfr* origin.

(iii) The third step involves genetic recombination proper, that is the series of events which lead to the *integration* of *Hfr* markers to form a recombinant chromosome.

(iv) The fourth step, or *expression*, comprises the events which, through segregation and phenotypic expression, extend from the formation of a

recombinant chromosome to that of a fully expressed recombinant bacterium.

Of these four main steps, the last two, integration and expression, are common to the different known processes of genetic transfer in bacteria, i.e. conjugation, transformation and transduction. Analysis of the results obtained in the study of bacterial conjugation involves the comparison, in each case, of the nature and extent of genetic transfer from donor bacteria to the zygotes with the subsequent integration of these characters to recombinants. The characters transmitted with high frequency by strain *HfrH*, the strain originally described by Hayes (1953*b*) are represented in Fig. 1. The order and relative distances of the characters located on this segment may be determined, as indicated in the legend, both by the genetic analysis of recombinants and by the time at which these characters penetrate, during transfer, into the F^- recipient.



A	—	—	90	70	40		25	15
B	8	8½	9	11	18		25	26

Fig. 1. Genetic map of the segment injected with high frequency by the *Hfr* isolated by Hayes. The location of the different characters as measured in crosses *Hfr* × F^- by *A*: the percentage of $T^+L^+S^r$ recombinants which have inherited the different *Hfr* alleles; *B*: the time at which individual *Hfr* characters start penetrating into the F^- recipient in a Waring blendor experiment.

The mating systems of E. coli *K-12*

When an attempt is made to compare conjugation between *Hfr* and F^- bacteria on the one hand, and between F^+ and F^- bacteria on the other, the question arises as to whether the low frequency of recombination observed in the latter crosses is a consequence of a low frequency of conjugation, of transfer, or of integration. In $F^+ \times F^-$ crosses, the F^+ character itself is transmitted at high frequency to F^- cells (Cavalli *et al.* 1953) and this is also the case for the ability to produce certain colicins (Fredericq & Betz-Bareau, 1953). This indicates that the frequency of effective contacts, and hence of conjugation, is as high in $F^+ \times F^-$ crosses as in *Hfr* × F^- crosses (Jacob & Wollman, 1955), a prediction which is verified by microscopic studies (Anderson *et al.* 1957). Other lines of evidence support the hypothesis that low frequency of recombination is a consequence of a low frequency of transfer.

The question therefore arises of the origin of those recombinants which are formed at low frequency in $F^+ \times F^-$ crosses. They could either result from a low but constant probability for each F^+ cell to transfer the character under consideration—or from the presence, in F^+ cultures, of a small proportion of *Hfr* mutants of variable nature. Evidence for the validity of

the latter hypothesis comes from both the quantitative and qualitative results of 'fluctuation tests' as well as from the possibility of isolating, with a high yield, those *Hfr* mutants which are responsible for the formation of recombinants of any type in $F^+ \times F^-$ crosses (Jacob & Wollman, 1956*a*).

It may be pointed out that both mechanisms could play a part in the formation of recombinants in $F^+ \times F^-$ crosses. The demonstration that most of these recombinants, if not all of them, are formed by *Hfr* mutants present in the F^+ population, however, indicates that this mechanism is the prevailing one. If any recombinant were formed by F^+ donors, the detection of this latter mechanism would be extremely difficult, and there is, at the present time, no experimental evidence for its existence.

The patterns of chromosome transfer in Hfr *mutants*

The finding that most, if not all, recombinants formed in an $F^+ \times F^-$ cross are due to *Hfr* mutants has two main implications. On the one hand, the fact that any known marker of an F^+ may be transmitted to a recombinant, implies that there must exist *Hfr* mutants able to transfer such markers at high frequency. On the other hand, the fact that any *Hfr* strain, such as the strain of Hayes, may transfer at high frequency only a group of characters suggests that the different *Hfr* mutants present in an F^+ culture must differ as to the nature of the chromosomal segment they are able to transfer.

This is indeed what is found. Any known genetic character of *E. coli* K-12 may be transferred at high frequency by a given type of *Hfr* mutant. These mutants differ from each other in the nature of the chromosomal segment they are able to transfer at high frequency. No *Hfr* mutant has been isolated so far which can transfer at high frequency all the genetic markers known (Jacob & Wollman, 1956*a*).

When studying any *Hfr* strain, one may obtain, as shown in previous sections, two types of information. On the one hand, one may determine, by genetic analysis, which characters are transmitted at high frequency to recombinants as well as their relative frequency of transmission. On the other hand, one may determine, by a blendor experiment, the sequence in which these characters are transferred and the time at which any given character enters the recipient.

Such an analysis has been carried out with a variety of *Hfr* mutants isolated from different strains of *E. coli* K-12 (Jacob & Wollman, 1957). Preliminary results are summarized in Table 1. It is seen that different *Hfr* strains differ from each other not only in those *characters* that they are able to transfer at high frequency, but also in the *order* in which any group of characters may be transferred. The simplest representation of these results

consists in assuming that, for any given *Hfr* strain, a chromosomal segment is transferred through a given extremity O (the origin) and that it is the position of O which determines which characters are transferred at high frequency, as well as the order of their transfer. A remarkable feature of the genetic system of *E. coli* K-12 appears to be the existence of a predetermined pattern of arrangement of the genetic characters that an *Hfr* mutation will affect by determining the position of O and hence the orientation of transfer. When considering a given genetic character B, it is found to be linked to a character A on one side and to a character C on the other side, these linkage relationships being retained as long as O is not

Table 1. *Patterns of Hfr mutants*

Schematic representation of the characters injected with high frequency by some of the isolated *Hfr* mutants. Each line corresponds to an *Hfr* strain and the order of injection corresponds to the characters from left to right.

Type	O←
H	TL Az T_1 Lac T_6 Gal λ
1	L T B_1 M Mtol Xyl Mal S^r
2	T_1 Az L T B_1 M Mtol Xyl Mal S^r
3	T_6 Lac T_1 Az L T B_1 M Mtol Xyl Mal S^r
4	B_1 M Mtol Xyl Mal S^r λ Gal
5	M B_1 TL Az T_1 Lac T_6 Gal λ

located closer to B than either A or C. When B becomes linked to O on one side, either it remains linked to A, and then becomes completely unlinked to C, or it remains linked to C and then becomes completely unlinked to A. For instance, in an *Hfr* of type 5 (Table 1), the characters TL appear to be linked to M (methionine) on the one side and to Gal on the other side, the order of transfer being O-M-TL-Gal. In the classical *HfrH*, characters TL are now linked to O on one side and to Gal on the other side, the order of transfer being O-TL-Gal. On the contrary, in *Hfr* of type 2, TL are linked on one side to O, but on the other side they are linked to M and the sequence of transfer has now become O-L-T-M.

Such a relationship is valid for all the known characters of K-12 and for all the *Hfr* strains which have been isolated up to now, whatever the previous history of the F^+ strains of K-12 from which they originate.

When trying to draw a genetic map of K-12, one is thus faced with a paradoxical situation. On the one hand, we do not possess any direct information on the genetic system of either F^+ or F^- bacteria. On the other hand, when analysing any given $Hfr \times F^-$ cross, we can only determine a genetic map of the chromosomal segment transferred at high frequency by that *Hfr* strain. We are therefore compelled to compare the genetic segments thus determined. From such a comparison, the conclusion is reached that all the known genetic characters of *E. coli* K-12 are

linked, a conclusion which was already attained by the analysis of $F^+ \times F^-$ crosses (Clowes & Rowley, 1954; Cavalli & Jinks, 1956), but that they cannot be arranged along a straight line as is the case in most chromosomes. There is no reason in effect for interrupting the determined linkage group at any place preferentially to any other one, except when describing the properties of a particular *Hfr* strain. One is thus led to dispose all the

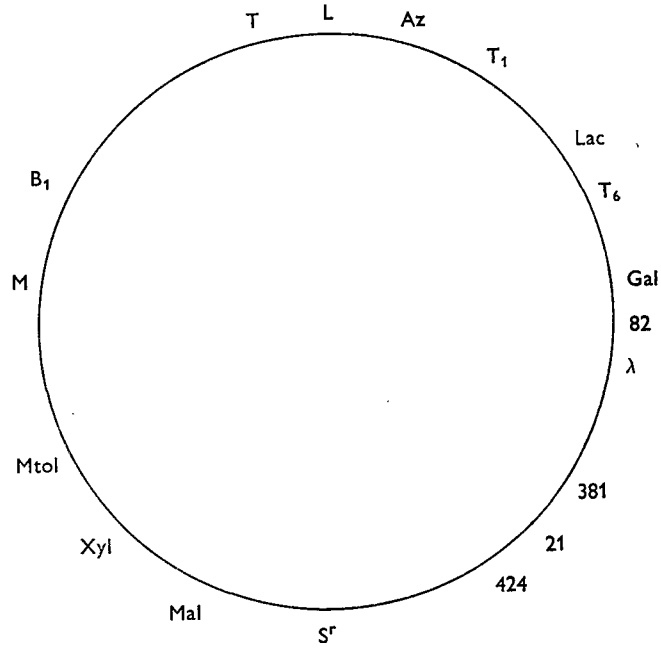


Fig. 2. Diagrammatic representation of the K-12 chromosome, as it results from a comparative study of the segments injected with high frequency by different *Hfr* strains. This diagram represents only the sequence of characters, not the distances between them. Symbols refer to threonine (T), leucine (L), methionine (M) and thiamine (B_1) synthesis; resistance to sodium azide (Az), bacteriophages T_1 and T_6, streptomycin (S); fermentation of lactose (Lac), galactose (Gal), maltose (Mal), xylose (Xyl) and mannitol (Mtol); ultra-violet inducible prophages 82, λ, 381, 21 and 424. (Possible mechanism of the $F^+ \rightarrow$ *Hfr* mutation: insertion of a specific factor at a given place of the circular linkage-group would result in the interruption of the circle. One extremity of the linkage group would behave as the origin. The other would carry the *Hfr* character.)

known genetic characters on a circle (Fig. 2). Since all *Hfr* mutants derive from F^+ strains, the underlying pattern of all *Hfr* would be that of the F^+ genetic system. It seems unnecessary to emphasize that this diagrammatic representation, which is the simplest one that will account for the observed results at the present time, is not meant to imply that the bacterial chromosome is actually circular.

Thus we have, on the one hand, in every individual *Hfr* a linear representation, one extremity of which is perfectly determined by the origin O,

and, on the other hand, a circular representation for the whole sequence. Under this scheme, the pattern of any existing *Hfr* may be obtained by interruption of the circle at the proper place, one of the extremities thus formed becoming the origin O.

Once the complete sequence of the K-12 linkage group has been determined by comparing the segments transmitted with high frequency by the various *Hfr* strains, one may analyse the capacity of a given *Hfr* type to transmit to recombinants those characters which appear only with low frequency. It seems that any *Hfr* type is indeed able to transmit all the known characters, but the frequency with which a given character is transmitted decreases the further from O this character is located in the particular *Hfr* strain. Such a genetic polarity may equally be interpreted as resulting from a gradient of pairing (Cavalli & Jinks, 1956) or from a gradient of transfer (Wollman & Jacob, 1957). The available evidence seems to indicate that the genetic polarity does reflect a polarity of transfer. The further from O a character is located, the greater the chance that a break will prevent its transfer during conjugation. When characters are located at a given distance from O, the probability for their transfer becomes so small that they appear very rarely among recombinants. These are the characters which are said to be transmitted to recombinants at low frequency in an $Hfr \times F^-$ cross.

Among characters transmitted with low frequency seems to be the *Hfr* character itself. Several of the isolated *Hfr* strains have been analysed for their capacity to transmit their *Hfr* character. With these strains, the recombinants having inherited from the *Hfr* parent the characters located close to O and transmitted with high frequency (proximal characters) are all F^-. On the contrary, among the few recombinants which have inherited from the *Hfr* parent characters located close to the extremity opposite to O (terminal characters), many are *Hfr*. When crossed with F^- bacteria, these *Hfr* recombinants appear to inject their markers in the same order as the one found for the *Hfr* parent from which they were derived. These properties of *Hfr* strains are similar to those already described for the two *Hfr* strains which were first isolated (Hayes, 1953*b*; Cavalli & Jinks, 1956). It appears, therefore, that the *Hfr* character does segregate among recombinants but that its linkage to other markers depends upon the strain considered. In any given *Hfr* strain, the *Hfr* character seems to be located at, or close to, the terminal extremity of the linkage group.

If confirmed by further investigation, this could be interpreted by the assumption that the event, which, in the $F^+ \rightarrow Hfr$ mutation, is supposed to result in the rupture of the circle at a given point, not only would determine the position of O at one of the two ends of the linkage group, but also the

location of the *Hfr* character at the other end. The properties of the different *Hfr* strains could thus be accounted for by the single hypothesis that the insertion of a specific factor at the proper place in the circular linkage group would determine the rupture of the circle. One extremity of the linkage group would behave as the origin O and would be injected during the process of mating. The other extremity would be terminal and would carry the *Hfr* character.

Again it must be clearly stated that this hypothesis provides only a formal model and that other schemes might well explain the experimental results. The proposed model, however, appears to be the simplest one that accounts for all the data. Although the nature of the postulated factor, whose insertion would be responsible for the breaking of the circle, remains unknown, it might well be conceived as being similar to the 'controlling elements' described in maize by McClintock (1956).

One must conclude that any *Hfr* may, upon conjugation, inject into the F^- recipient, a particular sequence of genetic characters in an oriented way. The distance between these characters may be determined by genetic analysis, when these characters are not very far apart. For distant characters, genetic analysis is grossly deformed by the increasing probability of breaks occurring, during transfer, between these characters. A more precise determination of the distances may be obtained by measurement of the time at which different characters penetrate the F^- recipient during conjugation.

II. EFFECT OF ULTRA-VIOLET LIGHT AND ^{32}P DECAY ON BACTERIAL RECOMBINATION

Radiation and disintegration of radioactive phosphorus are known to induce various types of cellular lesions and more particularly local alterations in the genetic material. In bacterial conjugation, whereas the F^- recipient contributes to the zygote both its genetic material and its cytoplasm, the *Hfr* donor appears to contribute genetic material but very little, if any, cytoplasm. Exposure of the *Hfr* donor to physical agents is therefore likely to affect only one of the genetic elements taking part in recombination. Lesions induced in the *Hfr* may affect either the transfer of genetic material or its integration in the zygote after transfer.

As shown in previous sections, only a segment of the *Hfr* chromosome may be investigated in a cross between a given *Hfr* strain and a recipient F^-. The following discussion will only be concerned with the segment injected at high frequency by the strain *HfrH* isolated by Hayes (Fig. 1).

When crosses are performed between *HfrH* $T^+L^+Az^sT_1^s\ Lac_1^+\ Gal_b^+\ S^s$

and *P678* $F^-T^-L^-Az^rT_1^r\ Lac_1^-\ Gal_b^-\ S^r$,* a chromosomal segment O-TL-Gal (Fig. 1) of the *Hfr* is transmitted at high frequency. The S^s marker not being transferred at high frequency, streptomycin may be used for eliminating the *Hfr* parent after mating. By plating the zygotes on suitable selective media, one may select, in the same experiment, recombinants which have inherited certain characters of the *Hfr* parent, either *proximal* to O, such as T^+L^+ which are close enough to be used together as selective markers (recombinants $T^+L^+S^r$) or distal to O such as Gal^+ (recombinants Gal^+S^r). One may also select those recombinants which have received the T^+L^+ as well as the Gal^+ characters of the *Hfr* (recombinants $T^+L^+Gal^+S^r$). In such an experiment, one may compare the frequencies with which these different types of recombinants are formed as well as their genetic constitution, that is the distribution among these recombinants of the genetic markers contributed by the *Hfr* parent.

The effect of ultra-violet irradiation

If *Hfr* donors are first exposed to ultra-violet light and then mated with non-irradiated F^- recipients, the irradiated *Hfr* lose their ability to form recombinants exponentially as a function of the dose of ultra-violet (Fig. 3). The capacity to form either $T^+L^+S^r$ recombinants or Gal^+S^r recombinants is lost at the same rate. This indicates that the transfer of the distal markers is not more reduced than that of the proximal ones. It is not known whether ultra-violet light may or may not prevent transfer, but if it does, it is not by reducing the size of the transferred piece.

On the contrary, the capacity to form recombinants which have received *both* extremities of the TL-Gal segment ($T^+L^+Gal^+S^r$ recombinants) is lost at a faster rate. One must, therefore, conclude that lesions produced in the *Hfr* parent before mating alter the process of integration occurring in the zygote after transfer. They decrease the probability of simultaneous integration of distant markers.

The difference observed between the slopes of the $T^+L^+S^r$ and $T^+L^+Gal^+S^r$ recombinants indicates that the fraction of the $T^+L^+S^r$ recombinants which have inherited the Gal^+ marker from the *Hfr* parent decreases as a function of the dose of ultra-violet. One may therefore expect to observe an alteration of the genetic constitution of the recombinants formed by irradiated *Hfr* parents. As shown in Table 2, the frequency with which unselected markers of the *Hfr* are present in both $T^+L^+S^r$ and Gal^+S^r recombinants is strikingly reduced by irradiation. This means that irradiation of the *Hfr* parent results in a loosening of the linkage observed

* Synthesis of threonine (T), leucine (L), fermentation of lactose (Lac), galactose (Gal), sensitivity (s) or resistance (r) to sodium azide (Az), to phage T1 and to streptomycin (S).

between the markers of the TL-Gal segment. In other words, the probability of a crossover occurring between two markers is increased by irradiating the donor.

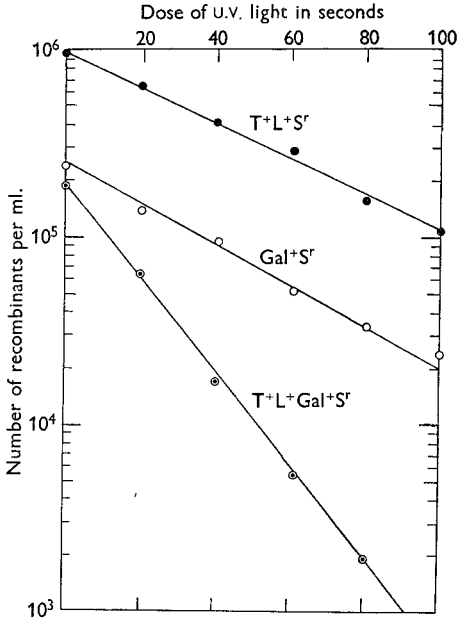


Fig. 3. Recombination between ultra-violet irradiated *Hfr* donor and non-irradiated F^- recipient. A suspension of *HfrH* is exposed to various doses of ultra-violet light. Samples of irradiated suspensions are mixed in standard conditions with non-irradiated *P678* F^- recipients and aliquots are plated on different selective media. The number of recombinants $T^+L^+S^r$, Gal^+S^r and $T^+L^+Gal^+S^r$/ml. of mating mixture are plotted on a logarithmic scale versus the dose of ultra-violet light in seconds.

Table 2. *Effect of ultra-violet irradiation on recombination*

Genetic composition of the recombinants formed in different crosses between *HfrH* and *P678* F^-. In the two last crosses, one of the parents was submitted to a standard dose of ultra-violet leaving about 30–50% survivors. The figures represent the percentage of recombinants $T^+L^+S^r$ or Gal^+S^r having inherited the characters Az, T_1, Lac and Gal of the *Hfr* parent.

	Genetic constitution of recombinants							
	$T^+L^+S^r$				Gal^+S^r			
Crosses	Az	T_1	Lac	Gal	TL	Az	T_1	Lac
Hfr × F^- control	91	72	52	29	74	78	73	74
Hfr U.V. × F^-	52	34	11	3	16	21	29	36
Hfr × F^- U.V.	88	60	35	16	41	49	57	62

Irradiation of the recipient also exerts some effect on the genetic constitution of recombinants, but to a much smaller extent than irradiation of the donor (Table 2).

This action of ultra-violet light on bacterial recombination appears to be similar to the effect which the same doses of ultra-violet exert on phage

recombination (Jacob & Wollman, 1955). Both effects can be best interpreted according to the hypothesis that, in bacteria as well as in phages, genetic recombination does not occur by breakage and reunion of complete strands but rather by a mechanism connected with replication, which, having commenced on one chromosome, would shift and be finished on the other (Levinthal, 1954).

Lesions produced randomly on the irradiated genetic material would interfere with the process of replication and therefore increase the frequency of the replication shifting from one parental chromosome to the other. This would result in a decrease of the size of the piece, or pieces, contributed by the irradiated parent to recombinants. Hence an apparent stretching of the linkage group.

The effect of ^{32}P *disintegration*

It is known that, like bacteriophages (Hershey, Kamen, Kennedy & Gest, 1951), bacteria containing ^{32}P of high specific radioactivity lose their viability as a function of ^{32}P decay (Fuerst & Stent, 1956). This method is of special interest since the lethal effects of ^{32}P decay cannot be accounted for by the ionizations produced, but appear rather to result mainly from 'short range' consequence of radioactive disintegrations, such as the transmutation $^{32}P \rightarrow {}^{32}S$ or the recoil energy sustained by the decaying P nucleus (Hershey *et al.* 1951; Stent, 1953). The favoured hypothesis is that the lethal effect of ^{32}P decay is due to those disintegrations which produce a rupture in the DNA chain.

This method may be applied to the study of bacterial recombination by mating ^{32}P-labelled *Hfr* donors with non-radioactive F^- recipients. By comparing the results of two types of experiments, it is possible to distinguish between the effects of ^{32}P decay on integration and its effects on transfer.

In one type of experiment radioactive *Hfr* donors are mated with non-radioactive recipients and ^{32}P decay allowed to occur *in the zygotes just after mating*, by storage in the cold. The capacity of the zygotes to give rise to recombinants is then measured as a function of ^{32}P decay. This type of experiment makes it possible to measure the effect of ^{32}P decay on *integration* independently of any effect on transfer.

The second type of experiment consists in allowing ^{32}P decay to occur *in the* Hfr *donors before mating*. Radioactive *Hfr* bacteria are stored in the cold and their *mating capacity*, that is their ability to transmit genetic characters to recombinants upon mating with F^- non-radioactive recipients, is measured as a function of ^{32}P decay. In this type of experiment radioactive disintegration may affect both the transfer of the genetic material and its integration (Fuerst, Jacob & Wollman, 1956, 1958).

(1) *The effects of* ^{32}P *decay occurring in the zygotes after mating.* When radioactive *Hfr* donors are mated with non-radioactive F^- recipients in non-radioactive medium and the early zygotes formed are stored in the cold, it is found that the capacity of these zygotes to form recombinants of any type decreases as a function of the time of storage, that is of ^{32}P decay (Fig. 4). This instability of the capacity of the early zygotes formed indicates that the genetic segment contributed by the *Hfr* parent remains susceptible to radioactive disintegration even after its transfer to the zygote. This is proof that the effects of ^{32}P decay are indeed a consequence of events occurring in the genetic material.

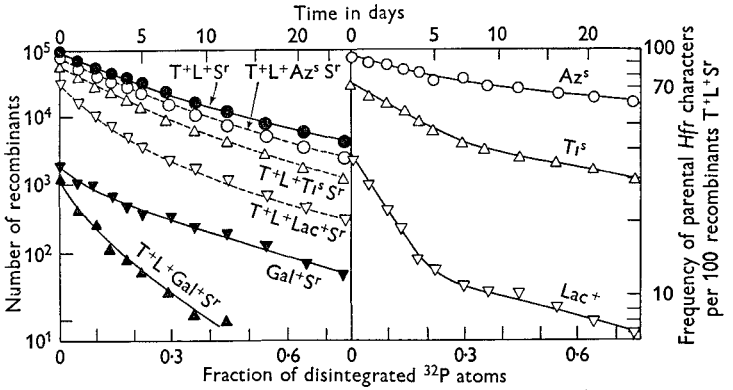


Fig. 4. Effect of ^{32}P decay occurring in the zygotes. Bacteria *HfrH* $T6S^s$ grown in a medium containing 110 mc./mg. of radio-phosphorus are washed, resuspended in buffer and mixed with an excess of non-radioactive *P678* F^- $T6^sS^r$, in a medium containing streptomycin, to prevent the *Hfr* parent from synthesizing nucleic acids. After 40 min. at 37°, KCN M/100 and an excess of phage T_6 are added to stop conjugation (Hayes, 1957). After 10 min. at 37°, the mixture is diluted in protective medium and samples are frozen in liquid nitrogen. Every day a sample is thawed and aliquots are plated on selective media.

The number of recombinants $T^+L^+S^r$, Gal^+S^r and $T^+L^+Gal^+S^r$ (left, solid lines) and the *proportion* of $T^+L^+S^r$ recombinants having one of the *Hfr* characters Az^s, $T1^s$, Lac^+ and Gal^+ (right) are plotted on a logarithmic scale versus the time in days and the fraction of disintegrated ^{32}P atoms. On the left figure are also plotted in dotted lines the *numbers* of recombinants $T^+L^+S^r$ having one of the *Hfr* characters Az^s, $T1^s$, Lac^+ and Gal^+, as calculated from the curves on the right.

When, in the same experiment, zygotes are sampled at different time intervals after mating, it is found that the capacity of the zygotes to form recombinants is the less sensitive to ^{32}P decay the later the time of sampling and that it becomes practically insensitive for the zygotes sampled after 120 min. This indicates that the genetic information carried by the injected chromosomal segment of the radioactive *Hfr* has been transferred to non-radioactive material.

Genetic analysis of the recombinants formed by early zygotes in the course of ^{32}P decay gives the following information. First of all, as may be

seen on Fig. 4, the number of recombinants which inherit either the T^+L^+ characters or the Gal^+ character of the *Hfr* parent decreases at about the same rate, whereas the number of those recombinants which inherit *both* the T^+L^+ and the Gal^+ characters ($T^+L^+Gal^+S^r$ recombinants) decreases at a much faster rate. These results, which are comparable to those obtained after ultra-violet treatment, indicate that the effect of ^{32}P decay in the zygote is to decrease the integration of the transferred genetic characters. Analysis of the $T^+L^+S^r$ recombinants shows that the linkage of the T^+L^+ characters to any of the unselected markers located on the TL-Gal segment of the *Hfr* donor such as Az^s, T_1^s, Lac^+ or Gal^+ is the more sensitive to ^{32}P decay the farther the marker is from T^+L^+. The initial slopes of the curves thus obtained depend on the order and relative distances of the characters located on the TL-Gal segment. These results suggest that ^{32}P disintegration destroys the integrity of genetic segments and that the sensitivity of any genetic segment is roughly proportional to its length.

It may be seen in Fig. 4 that the sensitivities of the linkages to T^+L^+ of the different markers decrease as a function of the time of storage and finally tend to a common slope. The explanation for this fact appears to be that the recombinants scored after a long time of storage correspond to multiple crossovers which simulate a reduction in size of the contribution of the *Hfr* chromosome.

(2) *The effects of* ^{32}P *decay occurring in the* Hfr *before mating.* When radioactive *Hfr* donors are stored and, after various time intervals, are mated with non-radioactive F^- recipients in non-radioactive medium, it is found that the capacity of these *Hfr* to form recombinants of any type decreases exponentially as a function of the time of storage (Fig. 5). However, their capacity for transmitting to recombinants the characters T^+L^+, *proximal* to O, decreases at a rate which is about one-third of the rate at which their capacity to transmit the *distal* Gal^+ character decreases. Moreover the capacity for transmitting *both* the T^+L^+ and the Gal^+ characters ($T^+L^+Gal^+S^r$ recombinants) is lost at the same rate as the capacity to transmit the Gal^+ character alone (Gal^+S^r recombinants). This suggests that when ^{32}P disintegration takes place in the *Hfr*, it affects not only the process of integration but also the transfer of genetic material from the donor to the recipient. Everything happens as though breaks had occurred on the *Hfr* genetic segment, certain *Hfr* cells still being able to transfer a proximal piece but not a distal one. Such an interpretation of the results is in agreement with the hypothesis according to which the lethal effects of ^{32}P decay result from interruptions on the DNA chain (Stent & Fuerst, 1955).

Analysis of the $T^+L^+S^r$ recombinants as to the presence of the genetic markers of the TL-Gal segment of the *Hfr* reveals that linkages $T^+L^+Az^s$, $T^+L^+T_1^s$, $T^+L^+Lac^+$, etc. are the more sensitive, the farther away the marker is, considered from the T^+L^+ selected characters (Fig. 5). By comparing the rates of survival to ^{32}P decay of the linkages considered, one may determine the order and relative distances of the markers. The fact, however, that the radioactive *Hfr* lose their capacity for transmitting both the T^+L^+ and Gal^+ characters together ($T^+L^+Gal^+S^r$ recombinants) at the same rate as they lose their capacity of transmitting the Gal^+ character alone (Gal^+S^r

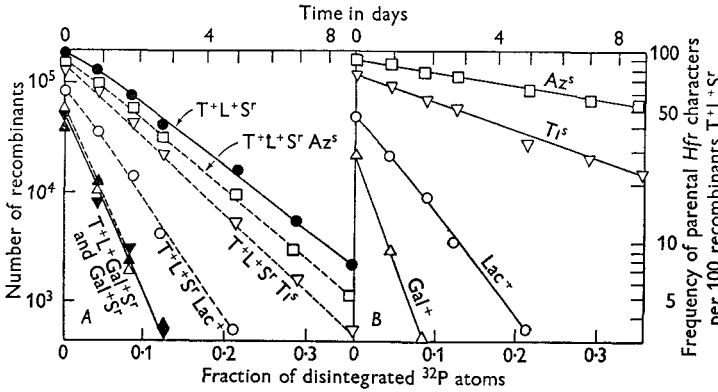


Fig. 5. Effect of ^{32}P decay occurring in the *Hfr* before mating. Bacteria *HfrH* grown in a medium containing 70 mc./mg. of phosphorus are centrifuged, washed and resuspended in protective medium. Samples are frozen in liquid nitrogen. Every day a sample is thawed, resuspended in buffer and mixed in standard conditions with an excess of non-radioactive recipient *P678* F^- ($2 \cdot 10^6$ *Hfr* and 10^8 F^-/ml.). After one hour at 37°, samples are diluted and aliquots are plated on selective media.

The number of recombinants $T^+L^+S^r$, Gal^+S^r and $T^+L^+Gal^+S^r$ (left solid lines) and the *proportion* of $T^+L^+S^r$ recombinants having one of the *Hfr* characters Az^s, T_1^s, Lac^+ (right) are plotted on a logarithmic scale versus the time in days and the fraction of disintegrated ^{32}P atoms. On the left figure are also plotted in dotted lines the *numbers* of $T^+L^+S^r$ recombinants having one of the *Hfr* characters Az^s, T_1^s, Lac^+ as calculated from the curves on the right.

recombinants), indicates that the slopes of the curves of Fig. 5 represent the rate of survival of the capacity of the *Hfr* to transmit any given character to a recombinant. Since these survival curves remain exponential over a long period, the mechanism of this loss in transmissibility of genetic characters appears to be mainly, if not exclusively, an exponential decrease of their transfer. The slopes of these survival curves would then be proportional to the distance of the various characters from the extremity O of the chromosomal segment. When the relative rates of decay of the individual markers are compared (T^+L^+ 0·35, Az^s 0·39, T_1^s 0·43, Lac^+ 0·67, Gal^+ 1), they are found indeed to be very similar to the relative times of entry of the same markers in a blendor experiment (T^+L^+ 0·34, Az^s 0·36, T_1^s 0·44, Lac^+ 0·72, Gal^+ 1).

Disintegration of radiophosphorus therefore allows the determination of the distances between genetic markers and the genetic maps which may thus be drawn of chromosomal segments transferred at high frequency by *Hfr* strains, are in every respect comparable with the maps, which may be determined on the one hand by genetic analysis and on the other hand by the time at which genetic characters are transferred in the course of conjugation.

III. DISCUSSION

Recombination of genetic characters in bacteria may be achieved by different processes which all involve the transfer of genetic material from a donor into a recipient bacterium. In the three known processes in which this genetic transfer occurs there is little doubt that the information is carried by DNA. In transformation, pieces of naked DNA are directly taken in from the culture medium into the recipient bacteria. In transduction, it is apparently a piece of bacterial DNA which has become included within the protein coat of a phage and which, together with the DNA of the phage, is injected into the recipient bacterium upon infection. In bacterial conjugation, the process of genetic transfer is accomplished by a more elaborate mechanism, which involves contact between bacteria of opposite mating types and the oriented injection of a chromosomal segment from the donor to the recipient. Non-chromosomal material, such as an enzyme or vegetative phage, has been found not to be transferred to any measurable extent during conjugation. Although such evidence is not definite, it suggests that, if there is any transfer of cytoplasmic constituents, it must be limited as compared with that of the genetic material. That this genetic material is nucleic acid is indicated by the effects on the formation of recombinants of ^{32}P disintegration occurring in the zygotes when ^{32}P-labelled *Hfr* is mated with non-radioactive recipients.

The most characteristic feature of bacterial conjugation is the progressive transfer of an oriented chromosomal segment of the donor which always injects the same extremity first into the recipient. The polarity of the transfer offers an opportunity of determining the position and distances of the genetic characters located on the chromosomal segment transferred by different independent methods. We will briefly compare the results obtained therewith.

Genetic analysis would allow an evaluation of the distances between characters in terms of frequency of recombination between such characters. However, the frequencies thus determined may vary according to the class of recombinants selected. This results both from the incomplete nature of the zygotes which contain the complete genome of the recipient and only

a fragment of the donor's, and from the variability in length of this fragment among different zygotes. By selecting recombinants for characters proximal to O (such as the $T^+L^+S^r$ recombinants obtained with *HfrH*), the recombination frequencies which may be measured (Fig. 1) would reflect both the real probability of recombination occurring between two characters and the heterogeneity in size of the segments transferred. Correction for this latter effect, which is formally comparable to a defect in pairing of homologous chromosomes (Cavalli & Jinks, 1956), may be obtained by different methods. One of them is to select those zygotes which have received chromosomal segments of equal length. This can be achieved by comparing normal crosses with crosses between lysogenic *Hfr* and non-lysogenic F^- where zygotic induction of the prophage selects those zygotes which have not received the prophage (Jacob & Wollman, 1956*b*; Wollman & Jacob, 1957). Another method consists in assuming a constant probability of breakage per length unit of the chromosome and determining the correction factor by trial and error. By combination of these two methods corrected values of the distances between genetic characters have been obtained which seem in good agreement with the results obtained with other methods (Jacob & Wollman, unpublished).

Blendor experiments permit a representation of the distances between genetic characters in time units by comparing their relative time of penetration into the recipient. The validity of this representation depends upon whether or not the transferred segment proceeds at a constant rate. Comparison between experiments done at different temperatures (Fisher, 1957), and even more the comparison between blendor and ^{32}P disintegration experiments indicate that this is the case for the characters located on the TL-Gal segment, that is along a segment which represents about one-third of the total chromosome length. The rate of penetration appears to decrease for characters located farther. It may be calculated that penetration of the whole chromosome at a constant rate would take about a hundred minutes.

Disintegration of radiophosphorus ^{32}P in the *Hfr* prior to mating appears to prevent the genetic markers from being transferred at a rate proportional to their distance from the origin O, that is to the number of phosphorus atoms existing between O and the marker considered. The genetic lesions provoked by ^{32}P decay appear, therefore, to be the direct consequence of the $^{32}P \rightarrow {}^{32}S$ transmutations which occur in the phosphodiester bonds which form the backbone of the nucleotide chain of DNA. On assuming that the efficiency of any radioactive disintegration is identical all along the chromosomal segment, a direct relationship may be established between the genetic and the physical structures of this segment. However, in order to evaluate

the distances between markers in terms of number of phosphorus atoms, one must know what is the efficiency with which ^{32}P disintegration prevents transfer. In the absence of any direct information on this efficiency, the assumption may be made, that it is the same as for the lethal effects in bacteria and in phage.

It becomes thus possible to compare the different measures of the chromosome of *E. coli* K-12. It is thus found that one minute in penetration corresponds roughly to about 20 corrected recombination units and to about 10^5 nucleotide pairs. This last figure is in agreement with an independent estimate of the DNA content of an *E. coli* nucleus, which would amount to about 10^7 nucleotide pairs, and of the time of transfer of the whole chromosome at a constant rate, which would take about 100 min. The three independent methods of measuring genetic segments in *E. coli* K-12 are therefore in reasonable agreement.

IV. SUMMARY

(1) Conjugation between *Hfr* donor and F^- recipient corresponds to the oriented transfer, under a rather precise time schedule, of a chromosomal segment from the donor to the recipient. The order and the distance between the characters located on the segment may be determined both by genetic analysis and by the time at which individual *Hfr* characters penetrate into the recipient.

(2) Various types of *Hfr* mutants may be isolated which differ by the characters they are able to transfer with high frequency and by the order in which the characters are transferred.

(3) By comparing the segments transferred with high frequency by various *Hfr* mutants, it is possible to determine a complete sequence of the known markers. Since no interruption can be observed, no extremity can be determined in the linkage group. One is thus led to dispose all the known genetic characters on a circle. In this model *Hfr* mutants would result from the insertion of a factor and the consequent interruption of the circle.

(4) Exposure of the *Hfr* donors to ultra-violet light before mating impairs integration of the characters located on the transferred segment. The number of genetic exchanges occurring between two given characters is increased by irradiation. This suggests that recombination in bacteria does not occur by breakage and reunion but rather by some 'copying choice' mechanism.

(5) If *Hfr* donors containing ^{32}P are mated with non-radioactive recipients, and if the mating process is interrupted early in order to allow

^{32}P decay to occur, the zygotes lose, as a function of time, their capacity to produce recombinants. Radiophosphorus disintegration appears to act by altering integration of the transferred *Hfr* markers.

(6) If radioactive *Hfr* are stored, in order to allow ^{32}P decay to occur before they are mated with non-radioactive recipients, they lose, as a function of time, their capacity to produce recombinants. Radiophosphorus disintegration appears to act by altering both transfer and integration of genetic material.

(7) It is possible to determine, by this method, the order and the distances between the markers located on the *Hfr* segment transferred with high frequency. The genetic map thus obtained does not differ from the map determined by other methods. It appears, therefore, possible to relate information on K-12 genetic material gained by genetic and by physical methods.

REFERENCES

ANDERSON, T. F., WOLLMAN, E. L. & JACOB, F. (1957). *Ann. Inst. Pasteur*, **93**, 450.

CAVALLI, L. L. (1950). *Boll. Ist. Sierot. Milano*, **29**, 1.

CAVALLI, L. L. & JINKS, J. L. (1956). *J. Genet.* **54**, 87.

CAVALLI, L. L., LEDERBERG, J. & LEDERBERG, E. M. (1953). *J. Gen. Microbiol.* 8, 89.

CLOWES, R. & ROWLEY, D. (1954). *J. Gen. Microbiol.* **11**, 250.

FISHER, K. W. (1957). *J. Gen. Microbiol.* **16**, 136.

FREDERICQ, P., & BETZ-BAREAU, M. (1953). *C.R. Soc. Biol., Paris*, **147**, 1653.

FUERST, C. R., JACOB, F. & WOLLMAN, E. L. (1956). *C.R. Acad. Sci., Paris*, **243**, 2162.

FUERST, C. R., JACOB, F. & WOLLMAN, E. L. (1958). *Ann. Inst. Pasteur* (in Preparation).

FUERST, C. R. & STENT, G. S. (1956). *J. Gen. Physiol.* **40**, 73.

HAYES, W. (1953*a*). *J. Gen. Microbiol.* **8**, 72.

HAYES, W. (1953*b*). *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 75.

HAYES, W. (1957). *J. Gen. Microbiol.* **16**, 97.

HERSHEY, A. D., KAMEN, M. D., KENNEDY, J. W. & GEST, H. (1951). *J. Gen. Physiol.* **34**, 305.

JACOB, F. & WOLLMAN, E. L. (1955). *C.R. Acad. Sci., Paris*, **240**, 2566.

JACOB, F. & WOLLMAN, E. L. (1956*a*). *C.R. Acad. Sci., Paris*, **242**, 303.

JACOB, F. & WOLLMAN, E. L. (1956*b*). *Ann. Inst. Pasteur*, **91**, 486.

JACOB, F. & WOLLMAN, E. L. (1957). *C.R. Acad. Sci., Paris*, **245**, 1840.

LEDERBERG, J. (1947). *Genetics*, **32**, 505.

LEVINTHAL, C. (1954). *Genetics*, **39**, 169.

MCCLINTOCK, B. (1956). *Cold Spr. Harb. Symp. Quant. Biol.* **21**, 197.

STENT, G. S. (1953). *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 255.

STENT, G. S. & FUERST, C. R. (1955). *J. Gen. Physiol.* **38**, 441.

TATUM, E. L. & LEDERBERG, J. (1947). *J. Bact.* **53**, 673.

WOLLMAN, E. L. & JACOB, F. (1955). *C.R. Acad. Sci., Paris*, **240**, 2449.

WOLLMAN, E. L. & JACOB, F. (1957). *Ann. Inst. Pasteur*, **93**, 323.

WOLLMAN, E. L., JACOB, F. & HAYES, W. (1956). *Cold Spr. Harb. Symp. Quant. Biol.* **21**, 141.