The mechanism of chromosome transfer mediated by various sex factors in Escherichia coli K12

by

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A thesis presented for the Degree of Doctor of Philosophy

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July 1971

Abstract

Many sex-factors or transmissible plasmids are capable of transferring donor chromosomal genes during conjugation with recipient bacteria. This chromosome transfer is believed to result from the interaction of the sex-factor with the bacterial genome to form some type of physical association between the two, linking the genetic material of the bacterium to the transfer mechanism of the sex-factor. The results of crosses performed using recombination-deficient (recA) donor strains suggest that the great part of chromosome transfer by the majority of transmissible plasmids is dependent on the functional integrity of the bacterial recombination (REC) system. Virtually all transfer is abolished when the donor strain is defective in the REC system as the result of a mutation in the recA gene. At the same time, there is an indication of an alternative mechanism of transfer which is apparently independent of the REC system.

Most plasmid-chromosome interactions are perfectly adequate for the transfer of donor chromosomal material but rarely result in the reciprocal genetic exchange necessary for the formation of Hfr-type donors.

Chromosome transfer can be stimulated both by U.V. irradiation and by new infection of donor strains, and it is proposed that this stimulation is due to the induction of a plasmid-specific function which promotes interaction with the bacterial chromosome, resulting in the transfer of donor chromosomal genes.

A simple model for chromosome transfer by autonomous plasmids is proposed where the plasmid-specific function would normally interact

with the bacterial REC system to join the genomes of the bacterium and the sex-factor in a non-reciprocal recombination event producing a structure capable of single-stranded chromosome transfer.

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CHAPTER 1

BACTERIAL CONJUGATION

Introduction

In 1946 Lederberg and Tatum described the transfer of genetic material between two K-12 sub-strains of Escherichia coli. The process was called conjugation and shown to be dependent on cellular contact between the conjugating bacteria (Lederberg & Tatum, 1946a.b; Davis, 1950). This discovery of recombination in E. coli was the outcome of a carefully planned study. It was based on the ability to select rare prototrophic recombinant bacteria from mixtures of auxotrophic mutant strains when plated on minimal agar. These recombinants arose at a frequency of 10^{-6}-10^{-7}. Genetic analysis of the recombinants revealed that the unselected markers segregated among the protrophs as if they and the selected markers were arranged in a single, linear linkage group (Lederberg & Tatum, 1946a.b; Lederberg, 1947; see also Rothfels, 1952).

The historical background and much of the early work on conjugation has been most clearly reviewed in treatises by Jacob & Wollman (1961a) and by Hayes (1964, 1968). Also, certain aspects of bacterial conjugation have been the subject of a number of reviews by Adelberg & Pittard (1965), Anderson (1968), Brinton (1965), Curtiss (1969), Cuzin, Buttin & Jacob (1967), Czerwinska (1966), Falkow, Johnson & Baron (1967), Fredericq (1969), Hayes (1966d), Kunicki-Goldfinger (1968), Meynell, Meynell & Datta (1968), Scaife (1967), Valentine, Silverman, Ippen & Mobach (1969), Watanabe (1967), Willetts & Broda (1969).

Section 1

Mating types

In his initial studies on recombination in bacteria Lederberg assumed that both parents were equal partners in a fusion process, the results being a fully diploid zygote. However, much more information about the nature of conjugation was gained from an examination of the physiology of the process rather than its genetic outcome.

1-1 The significance of sexual differentiation

The transfer of genetic information during conjugation is unidirectional. One parent is exclusively the recipient, in which the whole recombination process occurs and the zygote is formed. The other parent acts as a genetic donor and on completion of this task are dispensible (Hayes, 1952a.b, 1953a.b). This raised the question, what determined whether a cell is a donor or a recipient? Hayes (1953a) conceived the possibility that genetic transfer might be mediated by some kind of infectious vector which resided in donor cells. This was confirmed by the finding that although a mating mixture yields recombinants at a frequency of 10^{-6}, the majority of the recipient population may be converted to the donor state (Cavalli-Sforza, Lederberg & Lederberg, 1953; Hayes, 1953a.b; Lederberg, Cavalli & Lederberg, 1952) while only those sub-lines of *E. coli* K-12 which harbour this additional genetic entity, termed *F* or the sex factor for fertility, are donors. These donors or F^+ strains are capable of donating chromosomal material to sub-lines which lack *F*, that is F^- or recipient strain (Hayes, 1953a.b).

When F^+ donor bacteria are mixed with a great excess of recipient bacteria, the recipients are converted into F^+ donors faster than the bacteria multiply. This suggested that the sex factor

proliferates autonomously and more rapidly than the bacteria to which it has been transferred. The F factor spreads throughout a recipient population in an epidemic fashion (Cavalli-Sforza et al, 1953; de Haan and Stouthamer, 1963; Lederberg et al, 1952). A pedigree analysis of single, exconjugant, multinucleate recipient bacteria showed that all their progeny were donors; demonstrating that either many sex factors are transferred to each recipient or else the F factor multiplies in the recipient prior to division (Lederberg, 1958).

A genetic analysis of the recombinants sired in reciprocal crosses suggests that only incomplete or partial zygotes are formed, the recipient bacteria contributing an entire genome and the donor only a part. This in turn suggested that the genetic anomalies hitherto observed must reflect the mechanism of genetic transfer and not the occurrence of post-zygotic events. It is now generally accepted that the donor bacteria transfer only a partial genome to the F^- recipients.

1-2 Conjugal fertility factors

In F^+ X F^- crosses, recombinants inheriting donor chromosomal markers are sired at frequencies of 10^{-4} to 10^{-6} per donor cell (Adelberg & Pittard, 1965; Hayes, 1953b; Wollman, Jacob & Hayes, 1956). The probability of F transfer per donor cell is one. Most of the recombinants as well as a large percentage of the non-recombinant recipient population inherit the fertility factor (Cavalli-Sforza et al, 1953; Hayes, 1953a).

This property of infectious heredity of donor ability observed in F^+ X F^- matings (Hayes, 1953a.b); Lederberg, 1958) is also found in other donor systems. For example, the majority of colicinogenic

strains, Col$^+$, are capable of transferring, by conjugation, the Col$^+$ factor. This element determines the synthesis of colicin (Fredericq, 1954, 1957, 1969; Furness & Rowley, 1957). More rarely the Col factor can transfer chromosomal genes (Clowes, 1961; Ozeki & Howarth, 1961). In Col V$^+$ X Col$^-$ crosses the Col factor is transferred at frequencies approaching one and chromosomal genes at frequencies of 10^{-5} to 10^{-6} (Clowes & Moody, 1966; Kahn & Helinski, 1964, 1965; Macfarren & Clowes, 1967). In crosses mediated by Col Ib donors the frequency of transfer of Col Ib is generally 10^{-2} to 10^{-4} and for the transfer of chromosomal genes 10^{-7} to 10^{-9} (Clowes, 1961; Clowes & Moody, 1966; Monk & Clowes, 1964a; Ozeki & Howarth, 1961). The probability of Col Ib transfer to the recombinants is almost one.

Bacteria possessing genetic determinants specifying antibiotic resistances, R-factors, in association with a conjugal fertility factor, RTF are also donor strains. The R-factor is transferred at frequencies of 10^{-2} to 10^{-4} (Akiba, Koyama, Ishiki, Kimura & Fukushima, 1960; Anderson, 1968; Meynell & Datta, 1965, 1966a.b; Meynell, Meynell & Datta, 1968; Ochaiai, Yamanaka, Kimura & Sawada, 1959; Watanabe, 1963, 1967) and the chromosome at frequencies of 10^{-7} to 10^{-9} (Meynell & Datta, 1965, 1966a.b; Sugino & Hirota, 1962).

Generally, there is no unique polarity of chromosome transfer with these factors. Any genetic marker on the chromosome is transferred with an approximately equal, but low probability by some cells in the population soon after the commencement of mating (Hayes, 1953b; Kahn & Helinski, 1964; Macfarren & Clowes, 1967; Sugino & Hirota, 1962). The efficient transfer of these factors relative to the frequency of chromosome transfer led to the notion that fertility

factors were autonomously replicating cytoplasmic elements or plasmids (Fredericq, 1957; Lederberg, 1952; Watanabe, 1963).

1-3 Inter- and intrageneric conjugation

The interchange of genetic material by conjugation can occur between many members of the family Enterobacteriaceae: within the genera Escherichia (Bernstein, 1958; Boyer, 1966; Cavalli-Sforza et al, 1953; Furness & Rowley, 1957; Lederberg, 1951; Ørskov & Ørskov, 1961; Sasaki & Bertani, 1965); Vibrio (Bhaskaran, 1958); Salmonella (Mäkela, 1963; Sanderson, 1967; Zinder, 1960b); Enterobacter (de Graaf, Thieze, Bonga & Stouthamer, 1968) and Shigella (Akiba et al, 1960; Schneider & Falkow, 1964), and between Escherichia and some strains of Shigella (Ketyi, 1969; Luria & Burrous, 1957); Salmonella (Baron, Carey & Spilman, 1959a; Baron, Gemski, Johnson & Wohlhieter, 1968; Zinder, 1960a); Serratia (Falkow, Marmur, Carey, Spilman & Baron, 1961); Pasteurella (Ginoza & Matney, 1963; Martin & Jacob, 1962) or Proteus (Baron et al, 1968; Falkow, Wohlhieter, Citarella & Baron, 1964).

Conjugation also occurs in several species of Pseudomonas (Chakrabarty & Gunsalus, 1969; Holloway, 1955; Loutit & Marinus, 1968; Loutit, Pearce & Marinus, 1968; Stanisich & Holloway, 1969); and of Pasteurella (Lawton, Morris & Burrows, 1968a.b); and in one species of Rhizobium (Heumann, 1968).

Although most studies of conjugation have been made under laboratory conditions, genetic transfer can occur in the intestine of a mammalian host (Akiba, Koyama, Kimura & Fukushima, 1961; Ducluzeau & Galinha, 1967; Jones & Curtiss, 1969, 1970; Kagiwada, Kato, Rokugo, Hoshino & Nishiyama, 1960; Kasuga, 1964; Mitsuhashi, Harada & Hashimoto, 1960; Schneider, Formal & Baron, 1961).

Section 2

The surface differences between donor and recipient bacteria

Donor bacteria have a different surface charge to recipient cells. This is indicated by their agglutination in buffers at low pH and by a lower affinity for basic dyes (Maccacaro, 1955; Maccacaro & Comolli, 1956). The existence of an antigen, termed f^+, has been demonstrated only in those bacteria which harbour the F sex factor (Ørskov & Ørskov, 1960). The treatment of bacterial cultures with sub-lethal concentrations of periodate devirilizes Hfr and F^+ donor strains but the recipient capacity of F^- strains is unaffected (Sneath & Lederberg, 1961).

2-1 The male specific bacteriophages

An indication of further surface differences was the isolation of bacteriophages which can adsorb only to donor bacteria. These are listed in Table 1.

2-2 The female specific bacteriophages

There also exist bacteriophages which are specific or quasi-specific for F^- recipient strains, see Table 2.

2-3 The sex pilus

The basis for some of these differences between donor and recipient bacteria was discovered by Crawford & Gesteland (1964). They showed that the male-specific RNA phage adsorbed to filamentous structures on the surface of Hfr and F^+ donor bacteria. The structures were shown to be synthesized under the control of the F sex factor and were termed F-pili (Brinton, Gemski & Carnahan, 1964).

TABLE 1

Male specific bacteriophages

Phage	Nucleic acid	Specificity for sex factor	References
f2	RNA	F	Löeb (1960)
MS-2	RNA	F	Strauss & Sinsheimer (1963)
R17	RNA	F	Paranchych & Graham (1962)
μ2	RNA	F	Dettori, Maccacaro & Piccinin (1961)
fcan	RNA	F	Davern (1964)
fr	RNA	F	Marvin & Hoffmann-Berling (1963)
M12	RNA	F	Hofschneider (1963)
QB[1]	RNA	F	Watanabe
f1[2]	DNA	F	Loeb (1960)
fd[2]	DNA	F	Marvin & Hoffman-Berling (1963)
M13[2]	DNA	F	Hofschneider (1963)
If1		Col Ib	Meynell & Lawn (1968)
If2		Col Ib	

1. Immunologically distinct from the other RNA male specific phages
2. Single stranded DNA

TABLE 2

Female specific bacteriophages

Phage	Nucleic acid	References
ø1	DNA	Dettori, Maccacaro & Piccinin (1961)
T3	DNA	Schell, Glover, Stacey, Broda & Symonds (1963)
T7	DNA	Makelä, Makelä & Soikkeli (1964)
øII	DNA	Cuzin (1965)
W-31	DNA	Watanabe & Okada (1964), Makura & Hirota (1961)
Tau	DNA	Hakura, Otsuji & Hirota (1964)
øH	DNA	Molnar & Lawton (1969)

The donor-specific DNA phages also adsorb to the F-pili, but only to the tip of the pilus (Caro & Schnös, 1966). A great deal of work has been done on the structure and function of the F-pilus (Brinton, 1965, 1967; Brinton & Beer, 1967).

In addition to the F pilus there exists another type of sex pilus, called the I-pilus. This is produced by cells harbouring either Col Ib or Rfi⁻ factors (Lawn, Meynell, Meynell & Datta, 1967). The I pilus is usually only produced during the temporary derepression of the plasmid's fertility system either by new infection or by U.V. irradiation. They are also produced by fertility-derepressed mutants of these plasmids. A very clear analysis of the relationship between the sex factor and the control of regulation of fertility has been made (Frydman & Meynell, 1969; Frydman, Cooke, Meynell & Meynell, 1970; Meynell & Aufreiter, 1969a.b.; Lawn et al, 1967; Meynell & Cooke, 1969; Meynell & Datta, 1967; Meynell, Meynell & Datta, 1968). The work of the Meynells indicate that sex factors in general specify either an F-like or I-like sex pilus, which may be subdivided serologically (Lawn & Meynell, 1970).

2-4 An analysis of the F transfer system

Nearly all the bacterial sex factors studied so far determine the synthesis of a particular type of sex pilus. The sex pilus is essential for successful conjugation and chromosome transfer (Brinton, 1965; Meynell, Meynell & Cooke, 1968; Novotny, Raizen, Knight & Brinton, 1969). Conjugal transfer is abolished or markedly reduced if the synthesis of the sex pilus is repressed or

in some way defective. Donor strains which are defective in sexual transfer can be isolated by selecting Hfr, F' or F^+ donors which are resistant to one of the male specific bacteriophages (Achtman, Willetts & Clark, 1968, 1971; Cuzin, 1962; Cuzin & Jacob, 1965, 1967; Hirota, Fujii & Nishimura, 1966; Nishimura, Ishibashi, Meynell & Hirota, 1967; Ohtsubo, 1970; Ohtsubo, Nishimura & Hirota, 1970). The majority of these defective donor strains are resistant to all the male specific bacteriophages. They produce no observable sex pili, as judged by electron microscopy. These mutant donors are believed to be defective in sex pilus synthesis and have been termed spi^- (Cooke, Meynell & Lawn, 1970). However, some of these mutant sex factor do produce sex pili and absorb some of the donor specific phages. They are perhaps best called tra mutants for transfer-deficiency (Achtman et al, 1971; Ohtsubo, 1970; Ohtsubo et al, 1970). Many tra mutants of an F'lac factor have been isolated and characterised (Achtman et al, 1968, 1971 and to be published). The properties of some mutants are shown in Table 3. They have been assigned to ten complementation groups (Willetts & Achtman, 1971 and to be published). They have been ordered on a genetic map of an F'lac factor by complementation studies, using defective Hfr strains with overlapping deletions into the integrated sex factor (Ippen, Achtman & Willetts, to be published), see Figure 1.

TABLE 3

The properties of some transfer-deficient F'lac mutants[a]

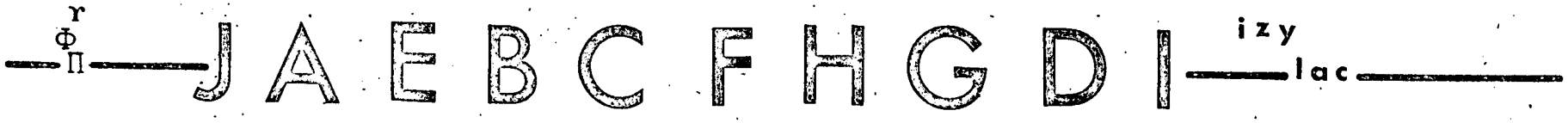
F prime[b]	tra mutation	sensitivity to phage f1	sensitivity to phage f2	Lac$^+$ progeny per 100 donor bacteria
JCFL0	tra$^+$	S	S	145
JCFL1	tra A1	R	R	$< 5 \times 10^{-5}$
JCFL2	tra B2	R	R	$< 5 \times 10^{-5}$
JCFL33	tra C33	R	R	$< 5 \times 10^{-5}$
JCFL83	tra D83	S	R	5×10^{-4}
JCFL18	tra E18	R	R	$< 5 \times 10^{-5}$
JCFL13	tra F13	R	R	1×10^{-4}
JCFL42	tra G42	S	S	$< 5 \times 10^{-5}$
JCFL55	tra H55	R	R	1×10^{-4}
JCFL65	tra I165	S	S	$< 5 \times 10^{-5}$
JCFL90	tra J90	R	R	1×10^{-4}

a. After Willetts (1970)

b. F'lac factors called JCLn series, derived from F42

Figure 1.

A map of the transfer genes of an F'lac factor.



Achtman, Willetts & Clark (1971),

Ippen, Achtman & Willetts (to be published),

Willetts & Achtman (1971),

Willetts (pers. commun.)

Section 3

The physical properties of conjugal fertility plasmids

The F sex factor and several F' factors were shown to consist of DNA by virtue of their incorporation of ^{32}P and the effect of mitomycin C in blocking this incorporation (Driskell & Adelberg, 1961). The size of the F factor was estimated as about 1-3% of the bacterial chromosome from ^{32}P inactivation studies. Similarly, it has been shown that the transfer of the F factor during conjugation is associated with the transfer of 0.5 to 0.9% of the total radioactively labelled thymine or thymidine present before mating. This is presumed to be DNA (Herman & Forro, 1962; Silver, Moody & Clowes, 1965). A more direct approach to the physical analysis of plasmids was to transfer the plasmid to a bacterial host with a different G + C context. If the G + C context is sufficiently different from that of E. coli (G + C = 50%), for example Serratia marcescens (G + C = 58%) or Proteus mirabilis (G + C = 39%) the DNA of the sex factor can be readily distinguished as a satellite band in a caesium chloride density gradient (Falkow et al, 1961; Falkow et al, 1964; Marmur, Rownd, Falkow, Baron, Schildkraut & Doty, 1961). The technique of using a different host bacterium has been superseded by use of a new dye. The direct centrifugation of crude lysates of donor strains in ethidium bromide caesium chloride gradients gives efficient separation of the plasmid DNA (Bazaral & Helinski, 1968; Kontomichalou, Mitani & Clowes, 1970; Nisioka, Mitani & Clowes, 1970). Ethidium bromide

intercalates between the bases of long, large DNA molecules, and decreases its density. The uptake of this dye is decreased when the DNA molecule is short and has a covalently closed, circular configuration. Since sex factors exist as covalently closed circles (Nisioka et al, 1970) they can therefore be separated from the DNA of their hosts' chromosome, even though both types of molecule have a similar G + C content. The physical measurements of some transmissible plasmids are shown in Table 4.

TABLE 4

Physical properties of some sex factors

Sex factor	Molecular weight doltons x 10^6	Contour length µM	References
F	45		Freifelder (1968)
	59	31	Clowes (pers. commun.)
	52		Falkow & Citarella (1965)
Rfi$^+$	69	28	Nisioka, Mitani & Clowes (1969)
R1	65		Silver & Falkow (1970a)
RTF	50		Silver & Falkow (1970b)
Rfi$^-$	35	18	Nisioka et al (1969)
Col VB trp cys	107	54.5	Hickson, Roth & Helinski (1967)
Col Ib	61	32	Clowes (pers. commun.)

Appendix 1

See the attached publication

Limits on Material Transfer during F^+ X F^- Matings in Escherichia coli K12

S. D. Silver, E. E. M. Moody & R. C. Clowes

J. Mol. Biol. (1965), 12, 283-286

The results of Borek & Ryan (1960) on indirect induction of phage λ, and of Fisher (1962a.b) on the conjugal transfer of immunity to phage λ suggested that there might be cytoplasmic transfer in F^+ X F^- crosses, but not in Hfr X F^- crosses. The experiments described by Silver, Moody & Clowes were designed to detect the limits of transfer of DNA, RNA and protein during F^+ X F^- crosses. The transfer of DNA was detected and this probably represents the transfer of the F sex factor. This would be sufficient to produce the Borek-Ryan effect (Monk, 1967, 1969).

The interpretation of Fisher's data suggesting the transfer of information in the absence of significant material transfer, perhaps a change in the conformation of the membrane is possibly correct. Fisher (pers. commun.) has since found that the effect he observed is not phage specific. Therefore is not ascribable to transfer of repressor molecules.

We demonstrated that 0.06 ± 0.19% of the radioactivity labelled protein present in F^+ donors was transferred to F^- recipients during conjugation. This result has been confirmed by

ol. Biol. (1965) **12**, 283–286

Limits on Material Transfer during $F^+ \times F^-$ Matings in *Escherichia coli* K12

revious studies on material transfer during bacterial matings, variable amounts NA transfer were measured (depending on the mating system and conditions) ve failed to detect any RNA or protein transfer, and could only establish upper s on such transfer (Silver & Ozeki, 1962; Silver, 1963).

ıce the results of the physiological experiments of Borek & Ryan (1960) and r (1962*a*,*b*) might suggest that cytoplasmic transfer occurs only in F^+ with F^- ıgs and not in matings with Hfr type males, we have extended our experiments donors.

ain we observed DNA transfer but no transfer of RNA or protein. These results led us to a novel interpretation of Borek & Ryan's and Fisher's experiments, ving the transfer of *information* during mating in the absence of *significant* ial transfer.

e experimental procedure was that of Silver (1963) unless otherwise indicated. active materials were from the Radiochemical Centre, Amersham, Bucks. Log cells, both non-radioactive and labelled (denoted with an asterisk) with hymidine (for DNA), [^{14}C]uracil (for RNA) or [^{14}C]leucine or lysine (for protein), washed twice and resuspended in fresh broth at about 5×10^8 cells/ml. Mating ıres of 3 ml. each of radioactive donor and non-radioactive recipient were ated for 60 minutes at 37°C on a rotor at $33\frac{1}{3}$ rev./min. The cultures were fuged and resuspended in fresh broth.

ng duplicate samples, the recipient cells were disrupted by lysis-from-without a large excess of T6 bacteriophage (to which the donor strains had previously made resistant) in the presence of DNase. The lysates were filtered through ore filters and radioactivity both on the filters and in the filtrates was measured. adioactivity which had been transferred from donor to recipient during mating be released by lysis and could be measured in the filtrates.

trols were run to test (1) non-conjugal cell to cell transfer (F^+ *T6-r** $\times$ F^+ *T6-s*), ontaneous and phage-induced release from the resistant cells (F^+ *T6-r** plus broth) and (3) the release of radioactivity from phage-sensitive cells *6-r* $\times$ F^- *T6-s**).

e the input F^+ was streptomycin sensitive (*str-s*) and the recipient was omycin resistant (*str-r*), transfer of the *F* factor could be measured by isolating omycin-resistant colonies and testing for "maleness" by means of the male c phage, μ_2 (Dettori, Maccacaro & Piccinin, 1961). The number of *str-r* F^+ fter 60-minute matings usually exceeded the initial number of *str-s* F^+ added. as probably due to very efficient mating, cell division in both the *str-s* and $^+$ populations and secondary *str-r* $F^+ \times$ *str-r* F^- matings.

results of the experiments are shown in Table 1. The transfer of DNA panying the transfer of the *F* factor is of marginal significance. A "best would be about 0·29% of the total cellular radioactivity. Due to growth and

TABLE 1

Transfer of ^{14}C in mixtures of E. coli *K12 strains of various mating types*

Experiment	% ^{14}C released by phage				% ^{14}C conjugal transfer (T − 1 or 2)	tra
	(T)	(1)	(2)	(3)		
DNA (*thymidine*)						
1	0·65	0·35	——	93·5	0·30	
2	0·68	0·52	0·48	97·0	0·16	
3	0·67	0·61	——	96.0	0·06	
4	1·70	——	1·06	97·0	0·64	
					1·16	
					Mean 0·29 ± 0·22	
RNA (*uracil*)						
1	0·10	0·15	0·25	82·0	−0·05	
2	0·25	0·18	0·19	68·0	0·07	
3	1·20	1·33	——	91·0	−0·13	
4	0·30	——	0·13	——	0·17	
					0·06	
					Mean 0·02 ± 0·09	
Protein (*leucine/lysine*)						
1 (leucine)	0·28	0·39	——	28·5	−0·11	
2 (leucine)	0·17	0·29	0·00	38·0	−0·12	
3 (leucine)	0·24	0·53	−0·13	46·0	0·04 †	
(lysine)	−0·20	0·23	——	40·0	0·43	
					0·24	
					Mean 0·06 ± 0·19	

† Mean value of (1) and (2) subtracted.

(T) represents transfer experiments between 58–161 *str-s T6-r* F^{+*} and W1-51 *str-r T6-*

(1) measures non-conjugal transfer between 58–161 *str-s T6-r* F^{+*} and 58–161 *str-r T6-s*

(2) measures spontaneous and phage-induced release from phage-resistant cells (58–16 *T6-r* F^{+*} in broth).

(3) measures efficiency of release and detection of radioactivity from phage-sensitiv (58–161 *str-s T6-r* F^{+} × W1-51 *str-r T6-s* F^{-*}).

Mixtures in experiment 1 were incubated 15 min between second and third centrifug whilst those in experiments 2, 3 and 4 were not (see Silver, 1963). % F transfer = number *st* cells after 60 min mating divided by input number *str-s* cells × 100. Other technique calculations are as described in Silver (1963).

secondary infections, we cannot ascertain exactly how many radioactive *F* f were transferred, but this is probably not very different from one radioact factor per input cell. Since there are an average of 2·5 chromosomes in each ac growing *Escherichia coli* cell (Tomizawa, 1960), this corresponds to 0·7% chromosome, which compares with the previously published estimates of 0·5 to (Herman & Forro, 1964) and 1·3 to 3·7% (Driskell-Zamenhof & Adelberg, Herman & Forro (1964) found that the transferred radioactive *F* factor, contained less than 1% as much radioactivity as the uniformly labelled chromo was itself labelled in only one of the two DNA strands.

We can detect no significant transfer of RNA or protein within limits of 0·1% of the cellular content. This raises the question of how to account f results of Borek & Ryan (1960), Devoret & George (1964) and Fisher (196 Either (1) there is physiologically significant transfer of RNA and/or protein b

low the level of resolution in these experiments; (2) there is physiologically ïcant transfer but of material which does not include uracil, leucine or lysine aps the transferred DNA is itself the active agent; Monk & Devoret, 1964); or ıe physiological effects do not arise from actual transfer of material. There is no iment at the moment to distinguish between these possibilities, but we favour ast. It is difficult to conceive of random cytoplasmic transfer at a level below which would still be physiologically important. It is equally difficult to imagine cific mechanism for the transfer of repressors and inducers. On the other hand, are known biological processes which involve the transfer of information or of sical state from one cell to another, or from one part of a cell to another, in the ce of transfer of material. The induction of enzymes in *E. coli* has been suggested ke place at the level of the cell membrane (Nisman, Fukuhara, Demailly & , 1962; Bishop, Roche & Nisman, 1964). It seems likely that the induction age λ also takes place at this level (Jacob & Monod, 1962). The transmission of a cal state along artificial membranes, and membranes of animal cells are also known (Dawson & Danielli, 1952), and conjugation may result in changes in the rial surface analogous to those following bacteriophage infection (Doermann, Silver, 1965).

e change in physical state induced by conjugation could well be a polarized ss from male to female, and not from female to male, since the two participants such an unequal role in this process, and already differ in surface properties of ens (Ørskov & Ørskov, 1960) and in fimbriation or piliation (Brinton, Gemski rnahan, 1964).

s work was undertaken whilst one of the authors (S.D.S.) was in receipt of a Fellow- of the U.S. National Science Foundation. We are indebted to the Biochemistry rtment, National Institute for Medical Research, Mill Hill, for the use of their low- round gas-flow counter.

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ved 7 January 1965

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Rosner, Adelberg & Yarmolinsky (1967). They showed that 0.05 ± 0.05% of the β-galactosidase originally present in donor cells is transferred to F^- bacteria during mating. This corresponds to no more than two active β-galactosidase molecules per F factor present in the donor strain.

CHAPTER 2

THE INTERACTIONS OF PLASMIDS WITH THE BACTERIAL CHROMOSOME

The transmissible plasmids are autonomously replicating cytoplasmic elements. They can determine their own transfer and the transfer of chromosomal genes to F⁻ recipient bacteria. Therefore the plasmids must somehow be able to interact with the bacterial chromosome.

Section 1

The concept of the episome

1-1 The isolation of Hfr donor strains

Donor strains which gave high frequencies of recombinants in crosses with F⁻ recipients were isolated from the same F⁺ donor (Cavalli-Sforza, 1950; Hayes, 1953b). These new highly fertile donor strains were designated Hfr (for high frequency of recombination) and provided essential tools for the investigation of the conjugation process.

If, after mixing an Hfr donor culture with a culture of a polyauxotrophic F⁻ strain, samples of the mating mixture are taken as a function of time and violently interrupted by one of a variety of techniques (Hayes, 1955, 1957a; Low & Wood, 1965; Wollman & Jacob, 1955, 1958), it is found that all the cells in an Hfr population transfer their chromosomes to recipient bacteria in a unique, oriented sequential manner. For a particular Hfr strain there is a particular extremity, designated O (origine) which is always the first part of the donor chromosome to penetrate the recipient bacteria

(Wollman & Jacob, 1955, 1958). The frequency of inheritance of donor markers in the recombinants is higher if the marker is located near the beginning (O) of the Hfr chromosome and is lowest for a terminally located marker (Hayes, 1957a; Skaar & Garen, 1956; Wollman, Jacob & Hayes, 1956). Although certain Hfr markers are inherited at high frequency by the recombinants, most donor genes are inherited at very low frequencies.

The data from many crosses and especially from zygotic induction experiments indicates that there is a fixed probability per unit time, that conjugation will be spontaneously interrupted with consequent breakage of the chromosome during transfer. Thus proximally transferred markers are much more frequently found in the recombinants than are the distally transferred markers (Jacob & Wollman, 1961a; Taylor & Adelberg, 1960; Wollman et al, 1956). The probability of this spontaneous interruption of mating is 6.4 per cent per minute of transfer time. This probability is reported to be independent of temperature, growth medium and the Hfr donor strain (Wood, 1968), although de Haan & Gross (1962) found that the growth medium greatly influenced the interruption of mating. Unlike $\underline{F^+}$ donors, Hfr strains do not convert the recipient population to the donor state in mixed culture. Only those recombinants receiving a terminally transferred Hfr marker inherit donor ability (Wollman & Jacob, 1958; Wollman et al, 1956) and these recombinants are Hfr donors of parental type. Nevertheless, Hfr donors do revert to the $\underline{F^+}$ donor state. Therefore, they must retain the sex factor of the $\underline{F^+}$ strain from which they stemmed but in some non-transmissible form

(Cavalli-Sforza et al, 1953). These observations led to the notion that in Hfr donors the sex factor is somehow attached or integrated into the extremity of the donor chromosome which is transferred last.

1-2 The circularity of the E. coli chromosome

An analysis of recombinants sired by a number of independently isolated Hfr strains, in interrupted mating experiments, showed that different Hfr donors transfer different overlapping sequences of donor markers to recipient strains, a particular sequence being characteristic of all the cells in a particular Hfr isolate (Jacob & Wollman, 1957, 1958). Since the proximal and terminal markers of every Hfr could be shown to be linked in transfer by some other Hfr strain, these data were interpreted as indicating that the chromosome of the F^+ donor from which they were all derived must be circular. During the formation of Hfr strains, the F factor attaches or integrates into the bacterial chromosome at different sites. This imparts the unique direction and orientation of transfer characteristic of the Hfr donor isolated.

Using the many independently isolated Hfr donors in interrupted mating experiments with suitably marked recipient strains it has been possible to construct a comprehensive genetic map of the E. coli chromosome (Taylor & Thoman, 1964; Taylor & Trotter, 1967; Taylor, 1970). The length of mating time necessary to transfer successive intervals of the chromosome defined by numerous genetic loci, when totalled show that the minimum time of transfer for the entire chromosome under standard conditions (at 37^{o}) is about 90 minutes (Taylor & Thoman, 1964; Taylor & Trotter, 1967; Taylor, 1970).

1-3 Jacob & Wollman's hypothesis of the episome

A comparison of the properties of the F fertility plasmid and the temperate bacteriophage λ showed similarities of a unique type. The word episome was coined(Jacob & Wollman, 1958c) to describe all genetic elements showing these unusual properties. A special feature of episomes is that they may be propagated in one of two alternative states - either as an integral part of the bacterial chromosome or by autonomously replicating in the cytoplasm. An episome is therefore defined as a non-essential genetic element, additional to the normal chromosome complement of the cell, which is acquired and is transmitted by infection or by conjugal transfer and which can replicate either autonomously or as part of the chromosome.

The concept of the episome raised some important and interesting questions. If an episomic element can become attached to the bacterial chromosome what is the structure of a chromosome containing it? How does the episome become attached to or detached from the chromosome? Finally, how is the replication of the episome controlled when it is autonomous in the cytoplasm and what happens to this control mechanism after integration into the chromosome?

Several forms of episome/chromosome complex were envisaged to explain the nature of the physical association of the two structures (Bertani, 1958; Jacob & Wollman, 1961a). The two main types of model involved either synapsis, or insertion of the episome into the chromosome. However, it proved too difficult to specify the nature of a synapsis which would concomitantly predict the properties of the integrated episome.

1-4 The Campbell model for the integration of episomes

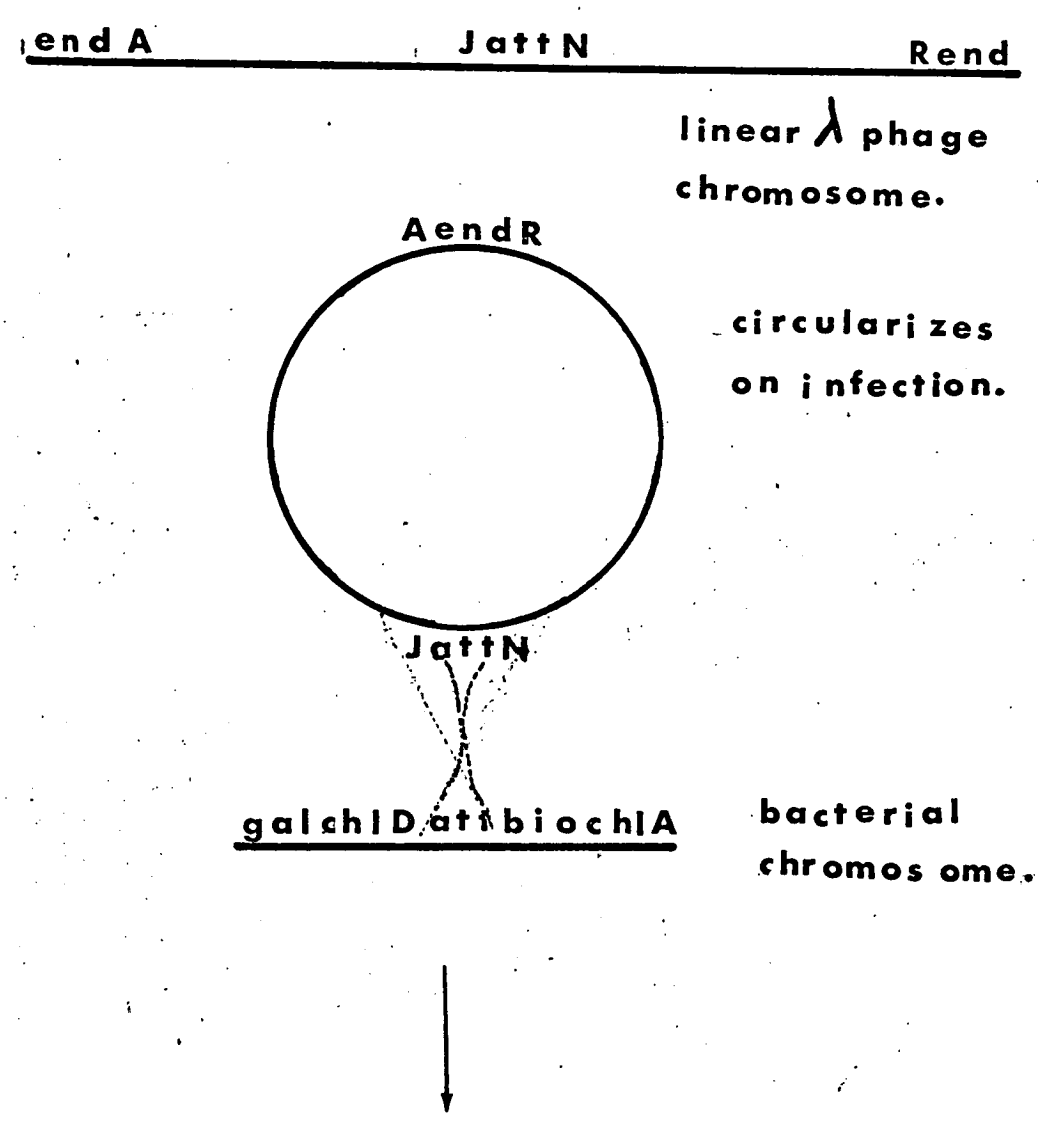
In 1962 Campbell proposed a specific insertion model for episomes, which had a high predictive value. This model has been extensively tested and is supported by much experimental evidence. It makes two basic assumptions; firstly, that the episome, like the bacterial chromosome, can exist as a circle, and secondly, that both the episome and the chromosome have special sites, regions of genetic homology, which permit a crossover to occur between the two genomes. Insertion thus occurs by a reciprocal genetic exchange between the two circles, to yield a single, continuous structure, as shown in Figure 2. According to the model, the integrated episome could regain its autonomy by a similar but reverse genetic exchange. If the same regions of homology are used as at the time of insertion, then a complete episome is released as a circle.

1-5 The isolation of F' donors

The existence of another type of donor, called an intermediate donor, was discovered by Adelberg & Burns, 1959, 1960). They observed that a particular Hfr donor strain transferred certain markers with only $^{1}/_{10}$th its former efficiency, while maintaining the same oriented sequence of transfer. Furthermore, this Hfr strain now transferred the donor state to recipient bacteria with a high efficiency. These converted recipients became intermediate donors and not F^{+} donors. The sex factor seemed to retain a memory of its original location on the Hfr chromosome. Treatment of the original intermediate donor strain with acridine dyes led to loss of the sex factor (Hirota, 1960) converting the strain into a recipient.

Figure 2.

A model for the attachment of the λ prophage.



after Campbell (1962).

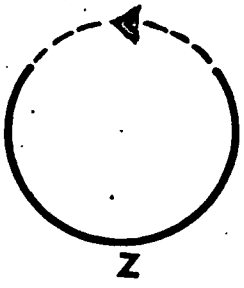
However, if this recipient strain was re-infected with a wild type sex factor from an F+ donor strain it became an intermediate type donor; the original Hfr location on the chromosome had evidently preserved an affinity for the sex factor. This was called an sfa or sex factor affinity locus (Adelberg & Burns, 1960; Richter, 1957, 1961). A wild type sex factor has a great affinity for this site, probably as the result of increased homology.

Logically this could be interpreted as the result of an exchange between the sex factor and the bacterial chromosome, whereby the chromosome retains part of the sex factor (sfa locus) while a sex factor carrying an adjacent part of the chromosome is released. This released factor was called an F-prime (F') or substituted sex factor.

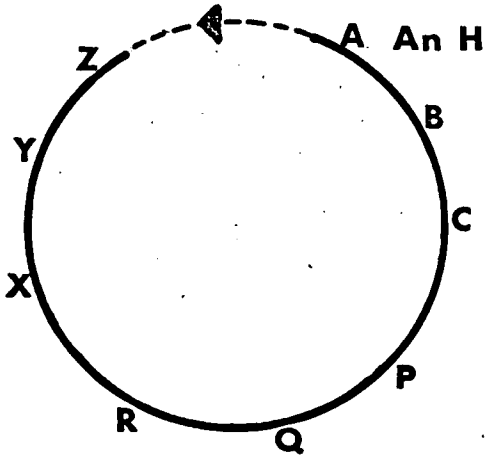
The observed interactions between the sex factor and the bacterial chromosome are certainly compatible with the Campbell (1962) model which provides a basis for linking the three types of donor strains which have been isolated, Figure 3. (Campbell, 1962; Gross, 1964; Scaife, 1967). The autonomous sex factor of an F+ donor is visualised as a circular structure which is opened during conjugation to allow its transfer in an oriented linear fashion (Matsubara, 1968; Vapnek & Rupp, 1970). Hfr and F' donors may be thought of as similar bacteria in which either the whole chromosome or merely a part of it is inserted into the sex factor, and are therefore transferred in a similar oriented linear way as part of the transferable factor.

Figure 3.

1.  An F sex factor.

2. 

An F' factor.

3. 

An Hfr chromosome.

A hypothesis to explain the relationship between the different donor types of E. coli K12.

Section 2

The circularity of episomes

The Campbell (1962) model requires that both the bacterial chromosome and the episome exist as, or assume, a circular structure prior to the reciprocal recombination event that brings about the integration of the episome into the chromosome.

2-1 The physical evidence for the circularity of episomes and plasmids

Autoradiographic analysis of tritium labelled DNA gently released from Hfr and F^- bacteria demonstrated that some chromosomes, at least, exist as continuous, closed Watson-Crick structures (Cairns, 1963a.b). Jacob & Wollman (1957, 1958b) predicted that the chromosomes of F^+ donor strain were circular as a result of their analysis of the recombinants sired by many different Hfr donor.

The entire genomes of the bacteriophage λ can be prepared from a suspension of the free phage particles (Hershey, Burgi & Ingraham, 1963). This phage DNA consists of linear molecules. They can be shown, *in vitro* to form new structures which are found to be circular under the electron microscope (Machattie & Thomas, 1964; Ris & Chandler, 1963). Additional evidence suggested that these linear molecules had end regions that were single stranded and complementary. It was concluded that the circular molecules are formed by pairing between the complementary nucleotides at the single stranded end regions of the linear molecules (Hershey & Burgi, 1965; Hershey et al, 1963; Kaiser & Inman, 1965).

By means of differential density gradient centrifugation,

circular λ DNA molecules have been detected shortly after the phage infects a sensitive bacterial host cell (Bode & Kaiser, 1965; Dove & Weigle, 1965; Saltzman & Weissbach, 1967; Young & Sinsheimer, 1964). These circular molecules cannot be opened up by heating. This implies that the ends are not merely held together complementary base pairing, but by covalent bonds. The Campbell (1962) model also predicts that circular molecules should appear when an episome returns to the autonomous state. Physical studies on the DNA isolated from an induced λ lysogen indicate that this is so (Lipton & Weissbach, 1966).

It has been possible to isolate and characterize the DNA of many Col and R factors by density gradient centrifugation after treatment with Ethidium bromide (see Chapter 1, Section 3). These DNA molecules of plasmids have been demonstrated, by electron microscopy to be circular (Hickson et al, 1967; Roth & Helinski, 1967; Nisioka et al, 1970). Circularity has been demonstrated for all the plasmids examined so far. This fulfils one of the Campbell (1962) model's requirements for integration into the chromosome.

Physical studies have shown that F' factors exist as covalently closed circular DNA molecules (Freifelder, 1968a.b). Genetic evidence suggests that the chromosome remaining after F' formation, although deleted for the information carried on the F' factor, has genetic continuity through the site of the deletion (Berg & Curtiss, 1967; Scaife, 1966).

2-2 The evidence for insertion of an episome into the chromosome

The Campbell (1962) model predicts that when an episome is inserted into the chromosome by a reciprocal recombination event, the genomes of the episome and the bacterium are continuous. Therefore, integration of an episome would increase the distance, and thus reduce the linkage between markers on either side of the integrated episome. This has been confirmed experimentally. For example, the linked markers *gal* and *bio*, lying on either side of the λ attachment site, are cotransduced by phage *P1* much less frequently when phage λ is integrated between them (Rothman, 1965). The genes of the λprophage can be cotransduced with the nearby chromosomal genes *gal* and *bio* (Rothman, 1965). The prophage ø80 has a similar effect on its neighbouring markers *supC* and *trp* (Signer, 1966). An elegant proof of the continuity of the bacterial and episomal genomes was the demonstration that deletions can arise which simultaneously affect both structures. Mutations to resistance to phage *T1* are often associated with a requirement for tryptophan and are known to be due to deletions extending into the adjacent *trp* region.

Occasionally some of these *tonB-trp* deletions are associated with a defective ø80 prophage (Franklin, Dove & Yanofsky, 1965). The ø80 attachment site is located on the opposite side of the T1 locus to *trp* and is very closely linked to it.

Hfr donor strains have been isolated with mutations at known chromosomal loci. These mutations were the result of the

integration of the F sex factor (Broda, pers. commun.; Curtiss, 1964; Richter, 1961; Schwartz, 1966). The F sex factor is cotransduced with chromosomal genes located near to the origin of an Hfr strain (de Witt & Adelberg, 1962). The cotransduction frequency of two chromosomal genes is greatly reduced when they are separated by an integrated F factor (Pittard, 1965).

CHAPTER 3

THE MECHANISM OF RECOMBINATION BETWEEN EPISOMES AND THE BACTERIAL CHROMOSOME

To what extent is the bacterial chromosomal recombination (REC) system involved in the reciprocal genetic exchange with episomes.

Section 1

Recombination in bacteria

1-1 The isolation of recombination-deficient mutants

Mutants of *E. coli* which are defective in the performance of recombination have been isolated by several methods (Clark & Margulies, 1965; Fuerst & Siminovitch, 1965; Howard-Flanders & Theriot, 1966; van de Putte, Zwenk & Rörsch, 1966).

Complementation analysis of these recombination-deficient (*rec*$^-$) mutations showed that they are recessive, and revealed that three genes or cistrons are involved: *recA*, *recB* and *recC*. The *recA* mutations are located between *pheA* and *cysC* (Willetts, Clark & Low, 1969); while *recB* and *recC* lie between *argA* and *thyA* (Emmerson, 1968; Willetts & Mount, 1969) on the standard map of *E. coli* (Taylor, 1970).

1-2 The properties of rec$^-$ mutants

Recombination-deficient (*rec*$^-$) mutants are defective in their ability to produce genetic recombinants by conjugation or by transduction. The *recB* and *recC* mutations produce a similar phenotype; namely a reduced but detectable level of recombination (Low, 1968). These mutants also have an increased sensitivity to ultraviolet light and a reduced breakdown of DNA following irradiation

(Howard-Flanders & Boyce, 1966). The reduction in the level of recombination in recA mutants is much greater, as is their sensitivity to U.V.-irradiation. Moreover, bacteria mutant at the recA locus degrade up to 30% of their DNA under normal growth conditions, and this degradation is greatly increased by U.V.-irradiation.

A mutation in either the recB or the recC genes leads to loss of an adenosine triphosphate-dependent deoxyribonuclease, ATP-DNase (Barbour & Clark, 1970; Buttin & Wright, 1968; Oishi, 1969). The products of the rec⁺B rec⁺C genes are responsible for the nuclease activity associated with genetic recombination (Clark, 1967). The rec⁺A product probably acts to control and reduce the breakdown activity catalysed by the rec⁺B rec⁺C nuclease (Buttin & Wright, 1968; Willetts & Clark, 1969). Whereas there is a significant residual level of recombination in recipients defective at recB or recC, no recA recipients form true recombinants (Low, 1968). This suggests that there could exist an alternative minor pathway of recombination, independent of the rec⁺B and rec⁺C products.

Revertant studies on recombination-deficient recB and recC mutants reveal at least two major genotypic classes. One class carries a revertant mutation which maps in or very near the recB and recC genes respectively and is probably due to a true back mutation or an intragenic suppressor mutation. It restores the ATP-DNase activity characteristic of the wild-type bacteria. The second class of revertants have mutations which are located far from the recB and recC genes and seem to be due to an indirect and non-informational suppression. These revertants have a high level of DNase activity

which is ATP-independent. The restoration of recombination proficiency by indirect suppression could involve the activation or derepression of one or a series of enzymes. These enzymes participate in a pathway of recombination, alternative to the recB and recC pathways (Barbour, Nagaishi, Templin & Clark, 1970), which is normally of minor importance. The ATP-independent DNase is probably one of the enzymes of this pathway.

Section 2

The recombination between episomes and the chromosome

2-1 The existence of genetic homology between the two genomes

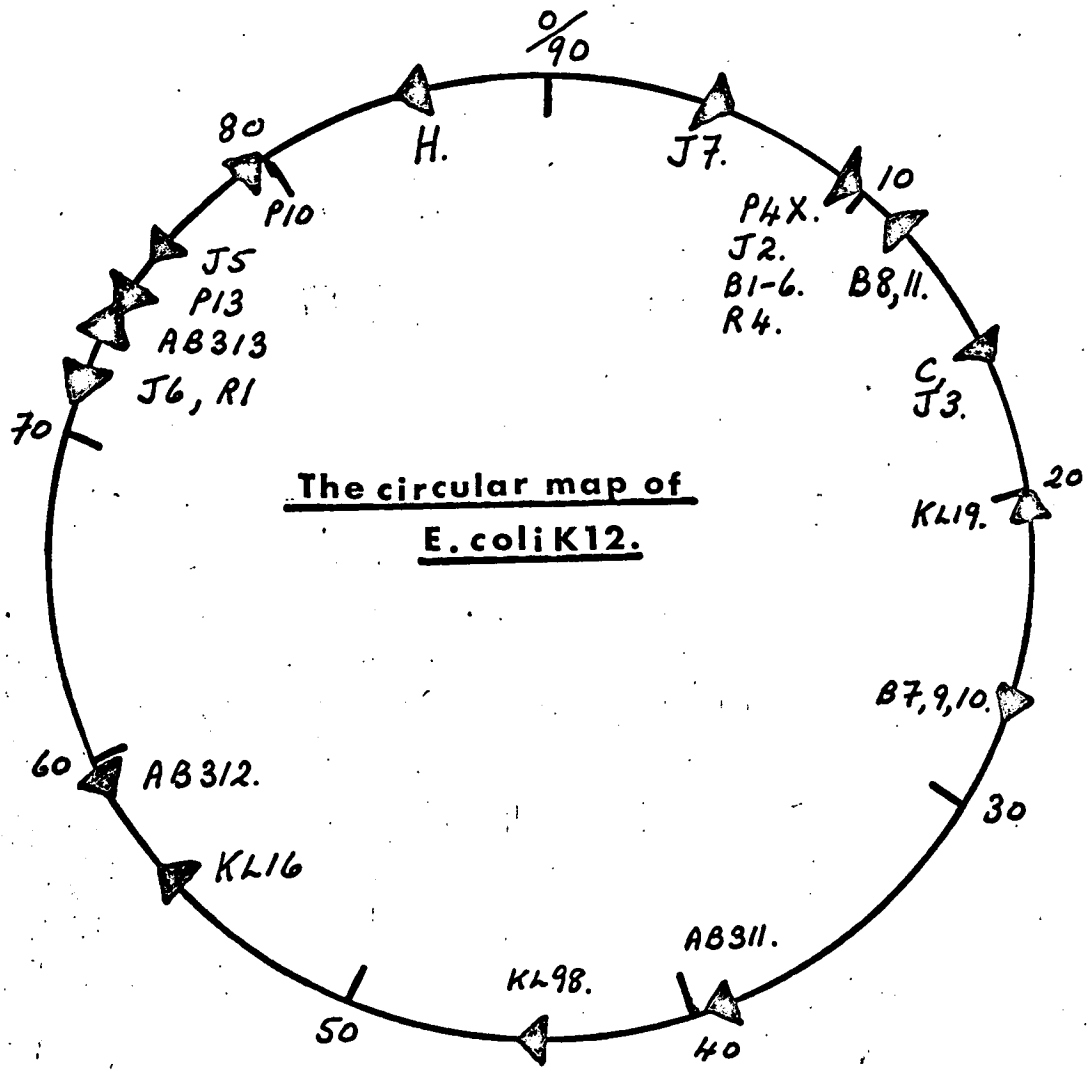
The direct proof of the existence of common base sequences in both the episomal and chromosomal DNA is not yet available although it is possible to form DNA-DNA hybrids between episomal DNA and E. coli DNA. Thus, the importance of genetic homology can only be assessed indirectly through the interaction of episomes with the chromosome.

All known episomes integrate into the chromosome in a non-random manner. This would indicate that genetic homology does indeed have a role in integration. The F sex factor, for which many integration sites are known, Figure 4, shows a distinct preference for certain locations on the chromosome (Broda, 1967; Matney, Goldschmidt & Erwin, 1963). If this interpretation is correct then an increased homology between the sex factor and the chromosome should increase the frequency of integration. It has been shown that F' factors integrate at a much higher frequency than the wild type sex factor (Adelberg & Burns, 1960; Hirota & Sneath, 1961; Jacob & Adelberg, 1964). These F' factors, as expected, integrate at the region

Figure 4.

The origines of some Hfr donor strains.

The sites of the sex factor's integrations.



AB311, AB312, AB313 (Taylor & Adelberg, 1960), B1-11 (Broda, 1967), C (Cavalli-Sforza, 1950), H (Hayes, 1953b), J2-6 (Jacob & Wollman, 1958b, 1961a), KL16, KL19, KL98 (Brooks Low, pers. commun.), P13 (Hirota et al, 1968), R1, R4 (Reeves, 1959).

homologous to that of the chromosomal fragment they carry. Conversely, when the homologous region is deleted from the chromosome, the efficiency of F' integration drops (Beckwith, Signer & Epstein, 1966; Cuzin & Jacob, 1964; Pittard & Ramakrishnan, 1964). However, deletion of the homologous region never completely eliminates integration of an F' factor. Rare Hfr derivatives can be isolated from such deletion F' strains. In these Hfr donors the F' factor is integrated at various sites on the chromosome (Broda, pers. commun.; Cuzin & Jacob, 1964; Moody, unpublished data; Scaife & Pekhov, 1964). The integration event, which is a genetic exchange, has been shown in some cases to involve the chromosomal fragment of the F' factor (Scaife & Pekhov, 1964).

Deletion studies with the bacteriophage λ indicate that the region interacting with the chromosome is less than that of an F'lac factor. Nevertheless, the probability of λ integration is much higher, up to 50% in one or two cell generations. This is a clear indication that genetic homology is not the only determinant in the λ prophage integration. Evidence will be discussed in Section 2-2, which indicates that the efficiency of lysogenisation depends on the synthesis of enzymes. These integration enzymes are phage-specific.

2-2 The episome-specific integration mechanisms

An important question to ask about episome integration is, what functions are provided by the bacterial host? Although, judging by the many Hfr donor strains isolated, the F factor does not integrate randomly into the E. coli chromosome, it clearly has a much lower chromosomal site specificity than the temperate phages λ and ø80.

Hfr donor strains may be isolated with opposite directions of chromosome transfer. This suggests that more than one site on the F factor can participate in integration events. Also, the genetic exchange leading to an F' factor's integration may occur between the chromosomal fragment and the bacterial chromosome. Consequently, if a single mechanism is employed, it does not require that the sex factor DNA takes part in the exchange event.

The integration of the temperate phages λ and ø80 involves specific sites on the bacteriophage and bacterial genomes. Presumably, for this reason, all of the many independently isolated lysogens of these phages are found to have the same prophage map. At least a part of the λ attachment site is lost in a deletion (b2) mutant of λ phage which covers 18% of the λ phage genome. These b2 mutants of λ phage cannot integrate into the chromosome on their own, but only if the chromosome already carries a λ prophage which provides homology (Campbell, 1965; Fisher-Fantuzzi, 1967; Jordan, 1964; Kellenberger, Zichichi & Weigle, 1960, 1961).

A possible explanation for the high efficiency of phage integration at their specific sites as compared with that of sex factor integration is that the phage mechanisms, although involving recombination, employ phage-specific enzymes (Scaife, 1967). In fact, the temperate phage λ has been shown to elaborate an enzyme which specifically catalyses recombination between the attachment sites (att) on the phage and on the bacterial genomes. This specific recombinase (integrase) functions so efficiently that the contribution to lysogenisation made by the bacterial recombinases is negligible

(Campbell, Adhya & Killen, 1969). Mutants of λ phage, which are unable to make the integrase enzyme (int⁻) have been isolated (Yarmolinsky, 1967; Zissler, 1967). These λint mutants are unable to attach to the chromosome with any appreciable frequency. They map outside the b2 deletion and their lost function can be provided by a λb2 mutant. The int gene will catalyse recombination between two λ phages as well as between the phage and the chromosome, but this recombination is restricted to the attachment regions (Gingery & Echols, 1968; Signer & Weil, 1968).

Additional information was obtained by examining the behaviour of both sex factors and known episomes, in bacteria which are deficient in their REC system, that is, the rec⁻ mutants of Clark & Margulies (1965) and Howard-Flanders & Theriot (1966). The behaviour of transmissible plasmids is described in Experimental Section 1. The efficiency of integration of most transmissible plasmids, as judged by their mobilization of chromosome, is drastically reduced in rec⁻ donor strains (Clowes & Moody, 1966), whereas the temperate phages λ and ø80 integrate normally into the chromosomes of rec⁻ mutants (Signer, 1968) suggesting they determine their own integration.

2-3 The pick up of genes by episomes

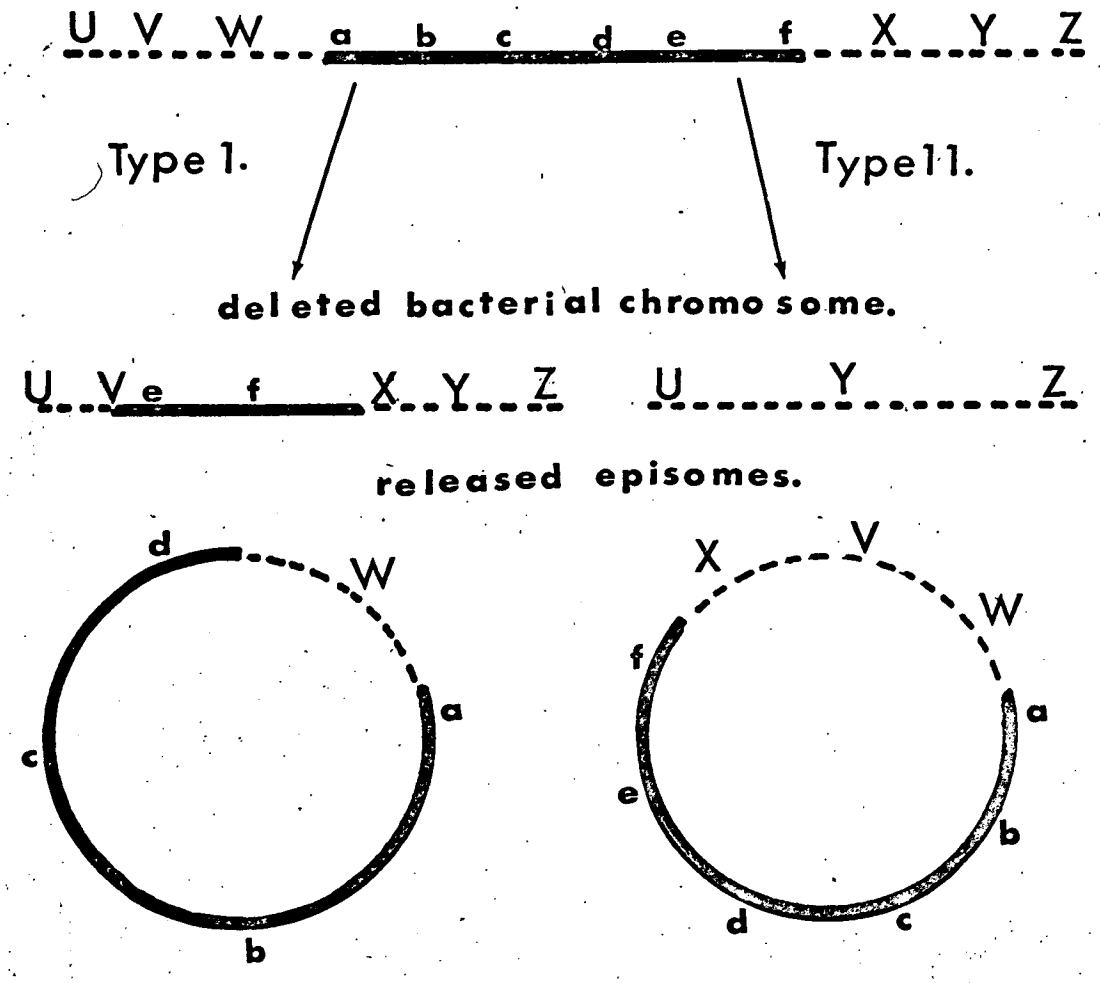
The specialized transducing phages, λdg and λbio, have picked up the gal and the bio genes respectively. These loci are situated at either end of the inserted λ prophage (Arber, 1958; Campbell, 1959; Morse, Lederberg & Lederberg, 1956a.b.). In a similar manner, an integrated sex factor may also pick up genes adjacent to its

integration site on reversion from the Hfr to the autonomous state (Adelberg & Burns, 1960). Many such varient sex factors have been isolated (see Scaife, 1967). The formation of these elements can be explained within the context of the recombination model for the insertion or the attachment of normal episomes from the bacterial chromosome (Campbell, 1962).

These substituted episomes would be formed by rare crossover events between sites that were not involved in the integration event (Figure 5). One of these sites must be located on the bacterial chromosome and the second site located either on the episome, or on the chromosome at the opposite side of the integrated episome. If the second site is within the episome, a crossover would release a circular element (Type 1) carrying a part of the chromosome but lacking certain episomal genes. Alternatively, if the second site is located on the chromosome, the structure formed (Type 11) would have all the genes of the episome and would contain bacterial chromosomal genes straddling the integrated episome (Scaife, 1967). All of the specialized transducing phage isolated from λ and Ø80 are Type 1 structures (Adler & Templeton, 1963; Campbell, 1959; Kayajanian & Campbell, 1966; Matsushiro, 1963). In transducing phage, there is clearly a selection for Type 1 structures, since Type 11 exchanges would result in larger than normal phage genomes, which probably could not be encased in a phage head. Also, the recombination system used for the formation of transducing phage probably demands the participation of phage DNA as at least one of the partners in the recombination event.

Figure 5.

The alternative types of abnormal excision of integrated episomes.



UVWXYZ are bacterial genes.

a b c d e f are episomal genes.

In a Type 1 excision, bacterial and episomal genes become fused by heterologous breaking and joining. A Type 11 excision only directly involves the bacterial regions.

2-4 The formation of F'factors

The formation of some F' factors has been studied in great detail. Both Types 1 and Type 11 structures have been found. The data suggests that the first F' factor to be isolated (F2) was formed by a Type I exchange (Adelberg & Burns, 1959, 1960). Another F' factor (F13) was formed by a Type 11 crossover event (Berg & Curtiss, 1967; Broda, Beckwith & Scaife, 1964; Jacob & Adelberg, 1959; Scaife & Pekhov, 1964). The Type II exchanges produce F' factors which are suitable for the direct genetic analysis of F' factor formation. The bacterial markers located on either side of the inserted sex factor, and transferred proximally and distally during conjugation, are linked on the F' factor, implying that it has circularized. In the bacterium in which a Type II F' factor arises, a segment of bacterial chromosome has been deleted and transferred to the autonomous sex factor. There is therefore no more homology between the F' factor and the chromosome than exists in an F^+ donor and, in fact, chromosome transfer occurs at low frequency just as in F^+ crosses. However, if essential genes have been incorporated into the sex factor, elimination of the F' factor by acridine orange will yield inviable cells and a small proportion of viable Hfr bacteria. When the latter are examined, the F' factor is found to have been re-inserted at many different chromosomal locations, and not at its original site. As is the case of Hfr strains derived from F^+ bacteria (see Chapter 2 Section 1), this provides evidence that the chromosome of the original F' cell is also circular.

In Type 1 exchanges part of the episome would be left in the chromosome, during the formation of the F' factor. It is reasonable to assume that a wild type sex factor would show a preference for such a site. The genetic homology of such a site with the F factor is much greater than with the remainder of the chromosome. The donor strain from which (Adelberg & Burns, 1959, 1960) isolated their F' factor F2, is of great interest. It harboured an autonomous episome which had a high affinity for a particular site on the chromosome of any normal F^- cell to which it was transferred. This preferred site corresponds to the location of the integrated sex factor in the parental Hfr strain. However, when the episome is removed from the strain in which it arose by curing with acridine dyes, this region of the resulting recipient strain still retains a great affinity for a wild type sex factor. The properties of these two products can be best explained by assuming that a Type I exchange occurred in the original Hfr donor strain. This exchange released an F' factor carrying some chromosomal material, but lacking some plasmid genes which remain at the site of the sex factor integration. This is a sex factor affinity, sfa locus (Adelberg & Burns, 1960; Richter, 1961).

Section 3

The role of the Campbell (1962) model in the transfer of chromosome by transmissible plasmids

The isolation of Hfr donors from F^+ strains, and the proof of the Campbell (1962) model (Broda, Beckwith & Scaife, 1964; Scaife & Pekhov, 1964) by means of the elegant analysis of the interactions

of F' factors with the chromosome recounted in the last section, caused it to be assumed that all transmissible plasmids which transfer the chromosome are episomes. That is, they integrate and transfer the donor chromosome in the same manner as F in an Hfr donor strain. In fact, the transfer of chromosome by an apparently autonomous cytoplasmic element became an immediate justification for classifying these elements as episomes.

3-1 The fertility of F^+ donors

In $F^+ \times F^-$ crosses, recombinants for chromosomal genes are sired at a frequency of 10^{-4}-10^{-6} per donor cell (Adelberg & Pittard, 1965; Hayes, 1953b; Wollman et al, 1956). It was proposed that the fertility of F^+ populations is due entirely to the formation of Hfr clones (Jacob & Wollman, 1956b, 1961a). This hypothesis was based on the results of fluctuation tests and the isolation of Hfrs from fertile cultures. However, Broda (1967), using non-selective methods concluded that the frequency and type of stable Hfrs isolated, could account for only about half of the observed inheritance of unselected markers. Similarly, Curtiss (1968), Curtiss & Renshaw (1965, 1969) claimed that only 10-20% of the fertility of an F^+ culture can be accounted for by the formation of stable Hfr clones. They also proposed that certain sub-lines of E. coli K-12, carrying the same F sex factor, gave positive fluctuation tests and others did not. Curtiss claimed that there were two evolutionarily distinct classes of K-12 sub-lines; Type I F^+ donor strains gave positive fluctuation tests and stable Hfr clones could be isolated from them, but not from Type II which gave negative fluctuation tests.

Therefore, the mobilization of the chromosome in Type II $\underline{F^+}$ strains, which occurs at a similar frequency to the Type I $\underline{F^+}$ donors, could not be due to the formation of stable Hfr strains. This also implies that chromosome transfer can occur by a method other than by the formation of stable Hfr clones. The clear distinction of $\underline{F^+}$ donors into Type I and Type II sub-lines has been questioned by Broda (pers. commun.) who has demonstrated positive fluctuation tests and isolated stable Hfr donors from supposedly Type II $\underline{F^+}$ strains.

The involvement of the bacterial REC system in the moblization of chromosomal genes by an F factor was examined by Clowes & Moody (1966). They concluded that the REC function was required for virtually all of the fertility of $\underline{F^+}$ donor cultures, after allowing for the slightly reduced efficiency of recA donor strains in conjugation.

This analysis of chromosome transfer has been greatly extended in Experimental Section 1.

Experimental Section 1

The materials and methods used in this section are described in the Appendices 5, 6 and 7.

The role of the bacterial recombination system (REC) in the transfer of chromosome by autonomous transmissible plasmids has been investigated. The ability of transmissible plasmids to mobilize the transfer of chromosome from recombination-proficient and -deficient donors was measured. The transmissible plasmids used are listed in Table 5.

TABLE 5

The source of the transmissible plasmids used in this study

Plasmid	Name or number of source strain	References
F	58-161 or W1655	Hayes (1952a) Lederberg & Lederberg (1953)
F'lac	W1655 F'lac	Scaife & Gross (1963)
Col V2	K94	Fredericq (1963) } Macfarren & Clowes (1967)
Col V3	K30	Fredericq (1963a.b.c) } Macfarren & Clowes (1967)
Col B1	CA18	Fredericq (1965) } Hausmann (1967)
Col B2	K77	Fredericq (1948) } Hausmann (1967)
Col B3	K166	Fredericq (1948) } Hausmann (1967)
Col B4	K98	Fredericq (1957) } Hausmann (1967)
Col VB	K260	Fredericq (1964, 1965)
Col Ib drd	Shigella sonnei P9	Ozeki, Stocker & Smith (1962), Ohki & Ozeki (1968)
R1 drd 19	J53 R^+ derivatives	Meynell & Datta (1967)
R192 drd F7	J53 R^+ derivatives	Meynell & Datta (1967)
R64 drd 11	J53 R^+ derivatives	Meynell & Datta (1967)
R144 drd 3	J53 R^+ derivatives	Meynell & Datta (1967)

1-A The efficiency of recombination-deficient strains as recipients in Hfr crosses

It is important in this analysis that the defective bacterial REC system is not complemented in the rec⁻ strains by the presence of any of the transmissible plasmids. The recipient capacity of these recombination-deficient, plasmid-carrying bacteria was measured in an Hfr cross, using Hfr Cavalli as the donor strain. Selection was made for the proximally transferred Hfr marker, proA. Results are shown in Table 7.

The control cross with the rec⁺ F⁻ recipient yielded between 5.0-15.0 x 10^{-2} pro⁺A strA recombinants. The frequency of recombinants issuing from the rec⁻ recipients is considerably lower; 1.0 x 10^{-5} for the recA F⁻ recipient and 2.0 x 10^{-3} for the recB F⁻ strain. The decreased ability of the rec⁻ strains to produce recombinants is further diminished by the presence in them of some of the plasmids. This decrease is due to the phenomenon of surface or entry exclusion since the plasmids convert the recipients into effective males, and is also shown by the rec⁺ donors. The crosses with the plasmid-carrying strains showing surface exclusion were repeated, using F⁻ phenocopies of them. In these crosses, the frequency of recombinants was found to be the same as in crosses using recA F⁻ or recB F⁻ strains as recipients. There is no restoration of the recombination-deficiency of either the recA or the recB strains by any of the plasmids.

TABLE 7

The efficiency of rec⁻ strains as recipients

The frequency of pro⁺A strA recombinants per 100 input Hfr⁺

Sex factor in recipient strain	rec⁺ donor as recipient	recA donor as recipient	recB donor as recipient
None	1.4×10^{-1}	1.0×10^{-5}	2.1×10^{-3}
F	2.0×10^{-4}	8.5×10^{-8}	1.3×10^{-6}
F'lac	1.8×10^{-4}	6.5×10^{-8}	1.6×10^{-6}
Col V2	8.0×10^{-2}	1.1×10^{-5}	1.1×10^{-3}
Col V3	1.6×10^{-1}	1.0×10^{-5}	1.7×10^{-3}
Col B1	6.0×10^{-2}	1.2×10^{-5}	2.4×10^{-3}
Col B2	1.6×10^{-1}	1.0×10^{-5}	1.8×10^{-3}
Col B3	7.0×10^{-2}	2.1×10^{-5}	3.0×10^{-3}
Col B4	5.6×10^{-2}	1.1×10^{-5}	1.0×10^{-3}
Col VB	1.8×10^{-1}	1.2×10^{-5}	1.8×10^{-3}
R1 drd 19	2.8×10^{-3}	1.6×10^{-6}	2.0×10^{-4}
R192 drd F7	4.0×10^{-3}	1.3×10^{-6}	1.8×10^{-4}
Col Ib drd	1.8×10^{-1}	1.0×10^{-5}	1.6×10^{-3}
R64 drd 11	4.8×10^{-2}	3.0×10^{-5}	2.0×10^{-3}
R144 drd 3	5.3×10^{-2}	2.0×10^{-5}	4.0×10^{-3}

Hfr Cavalli metB X AB1157 thr leu thi proA his argE strA F⁻

* At least 1,000 recombinants counted in each cross in several experiments.

1-B The transfer of extrachromosomal elements by recombination-deficient donor strains

The ability of recombination-deficient strains to conjugate and transfer their autonomous plasmids to recipient bacteria was examined. Most of the transmissible plasmids used in this study are fertility-derepressed mutants and are readily transferred to recipient strains. However, the colinogenic factors ColB1, ColB2 and Col B4 are fertility-repressed sex factors, and in order to measure their transfer it was necessary to prepare HFCT donor cultures (see Appendix 6D(i)(b)).

The results are shown in Table 8. There is a slight reduction in the level of plasmid transfer with some of the recombination-deficient donor strains. However, the extrachromosomal transfer from rec$^-$ donors is always greater than 75% of the rec$^+$ donor level. Where HFCT donor cultures were used the levels of plasmid transfer are somewhat variable, depending on the efficiency of the HFCT preparation. It was concluded that the recombinant-deficient donor strains were perfectly capable of making effective conjugal contacts with F$^-$ recipient bacteria and transferring their plasmids to these strains.

1-C The mobilization and transfer of chromosome by transmissible plasmids in recombination-deficient donor strains

In the initial studies with recA donors(Clowes & Moody, 1966) and with recB donor strains, the donor bacteria were derivatives of the recombination-deficient mutants AB2463 and AB2470 (Howard-Flanders & Theriot, 1966), see Table 6. These are multiply deficient

TABLE 8

Extrachromosomal transfer from rec⁻ donor strains

Percentage recipient population converted to donors*

Sex factor	rec⁺ donor	recA donor	recB donor
F	95	76	78
F'lac	98	74	79
Col V2	79	76	80
Col V3	82	84	81
Col B1	67	66	70
Col B2	74	78	76
Col B3	100	94	91
Col B4	84	80	86
Col VB	94	90	90
R1 drd 19	100	99	96
R192 drd F7	98	92	94
Col Ib drd	100	98	98
R64 drd 11	100	94	90
R144 drd 3	98	96	98

HFCT donor cultures of Col B1, Col B2 and Col B4 were used.

thr leu thi proA his argE strA X metB nalA F⁻

* Transfer measured by counting at least 1,000 infected recipient cells.

TABLE 6

The strains used to investigate the transfer of chromosome from recombination-deficient donor strains

AB1157

thr leu thi proA his arg E str A F^-

two recombination-deficient mutants isolated by Howard-Flanders & Theriot (1966)

AB2463

thr leu thi proA his arg E str A rec A F^-

AB2470

thr leu thi proA his arg E str A rec B F^-

These three strains AB1157, AB2463 and AB2470 were infected with the plasmids listed in Table 5.

auxotrophic strains and very limited in their usefulness as donors because of the restriction they place on the choice of a recipient. The recipient strain used to measure the donor ability of these *rec*⁻ donor bacteria was a 58-161 sub-line derivative. The *metB* mutation of this sub-line reverts to prototrophy at a frequency of less than 1.0×10^{-11} (Moody & Clowes, unpublished data). The recipient strain was also resistant to colicin Ib (i.e. *Col Ib-r*). This confers a partial cross-resistance to the group V and B colicins (Moody, unpublished data). The results obtained in the early crosses are shown in Table 9. The frequencies of *met*⁺*B strA* recombinants sired by the *rec*⁻ donors was lower than in the case of the corresponding *rec*⁺ donor strains. The *recA* donors show the greater deficiency, in fact the *recB* donors are only slightly deficient in their ability to transfer chromosome. Chromosome transfer from *recB* strains harbouring a newly transferred *F'lac* factor is only reduced to 50% of the *rec*⁺ level (Wilkins, 1969).

The interesting feature of these crosses was that, regardless of the frequency of *met*⁺*B* recombinants sired by the different *rec*⁺ donor strains, the frequencies produced by the *recA* donors are very similar. These frequencies range from 0.47 to 2.1×10^{-8}. This low level of chromosome transfer is reproducible and at least a thousand times higher than the measured reversion rate to *met*⁺*B*. This low level of residual transfer from *recA* donors is similar to the frequency of transfer by *Col Ib drd* donors. Chromosome transfer by *Col Ib* is apparently not affected by the recombination-deficiency of the donor strain. This is also true of the transfer by *R1 drd 19*,

TABLE 9

Chromosomal transfer from rec⁻ donors

The frequency of met⁺B recombinants*

Sex factor	rec⁺ donor	recA donor	recB donor
F	2.3×10^{-6}	1.2×10^{-8}	8.6×10^{-7}
F'lac	1.7×10^{-2}	2.1×10^{-8}	6.9×10^{-3}
Col V2	2.6×10^{-6}	6.1×10^{-9}	7.8×10^{-7}
Col V3	9.2×10^{-7}	1.0×10^{-8}	6.0×10^{-7}
Col B1	6.0×10^{-7}	8.6×10^{-9}	1.2×10^{-7}
Col B2	3.1×10^{-6}	4.7×10^{-9}	8.8×10^{-7}
Col B3	2.8×10^{-5}	2.0×10^{-8}	1.0×10^{-5}
Col B4	5.8×10^{-7}	7.6×10^{-9}	4.0×10^{-8}
Col VB	5.4×10^{-6}	6.0×10^{-9}	2.1×10^{-6}
R1 drd 19	2.0×10^{-6}	1.2×10^{-6}	6.6×10^{-7}
R192 drd F7	1.6×10^{-5}	4.9×10^{-9}	9.8×10^{-6}
Col Ib drd	9.1×10^{-9}	6.9×10^{-9}	9.0×10^{-9}
R64 drd 11	9.8×10^{-7}	5.6×10^{-9}	1.8×10^{-7}
R144 drd 3	1.1×10^{-7}	8.1×10^{-9}	8.8×10^{-8}

thr leu thi proA his argE strA X metB Col Ib-r strA

HFCT donor cultures of Col B1, Col B2 and Col B4 were used.

* Determined by counting at least 200 recombinants per cross

although the frequency of transfer is much higher than Col Ib. The results obtained in these early experiments served as a useful basis for further research. However, these early experiments suffered from two serious defects. In the first place the polyauxotrophic AB1157 derivatives were used as donors. Secondly, concentrated mating mixtures were plated in order to compensate for the low numbers of recombinants obtained in crosses with recA donors. This gives distorted results and inflated recombination frequencies due to considerable cell division and secondary mating on the plates.

In subsequent experiments the mating procedure was changed to that described in Appendix 6c(ii), an increased number of plates being used to score the small number of recombinants, and a new series of rec$^-$ donor strains being constructed in the W1655 sub-line as described in Table 10. These new donor strains were checked for their recipient capacity in an Hfr cross, using Hfr P13 as the donor strain (see Table 11). Their ability to conjugate and transfer their plasmids was measured and the results are shown in Table 12. The data were in agreement with the results obtained with the AB1157 derivatives. Since it was decided to concentrate on the effect of the recA mutation on chromosome transfer, no W1655 recB derivatives were made.

The advantages of the new W1655 derivatives as donors were immediately obvious. It was much easier to manipulate the culture conditions of these strains and many useful recipients became available to measure chromosome transfer. The W1655 rec$^+$ and recA donor strains were crossed with J62, proA his trp strA F$^-$

TABLE 10

The strains used to investigate the transfer of chromosome from recombination-deficient donor strains

A series of isogenic donor strains was derived as follows.

W1655 metB λ^- λ-r F^+	Lederberg & Lederberg (1953)
↓	
W1655 F^-	By acridine orange curing
↓	
W1655 nalA F^-	Isolated as resistant to 40 µg/ml nalidixic acid
↓	
W1655 nalA thyA F^-	By trimethoprim treatment
↓	
W1655 nalA thy^+ recA F^-	crossing with an Hfr JC5088

W1655 metB nalA F^- and W1655 metB nalA recA F^- were infected with all the plasmids listed in Table 5.

The recipient strain used was J62 proA trp his strA F^- derived from J62 F^+ Clowes & Rowley (1954)

TABLE 11

The efficiency of rec⁻ strains as recipients

The frequency of met⁺B recombinants per 100 input Hfr*

Sex factor in recipient	rec⁺ donor as recipient	recA donors as recipient
None	8.5×10^{-1}	2.1×10^{-5}
F	6.1×10^{-4}	3.4×10^{-8}
F'lac	3.0×10^{-4}	2.8×10^{-8}
Col V2	6.5×10^{-1}	3.2×10^{-5}
Col V3	9.0×10^{-1}	4.6×10^{-5}
Col B1	4.2×10^{-1}	1.2×10^{-5}
Col B2	2.8×10^{-1}	4.0×10^{-5}
Col B3	7.0×10^{-1}	3.1×10^{-5}
Col B4	6.6×10^{-1}	1.8×10^{-5}
Col VB	2.4×10^{-1}	1.8×10^{-5}
R1 drd 19	8.7×10^{-3}	4.1×10^{-7}
R192 drd F7	5.7×10^{-3}	6.0×10^{-7}
Col Ib drd	9.1×10^{-1}	3.7×10^{-5}
R64 drd 11	1.0×10^{-1}	1.0×10^{-5}
R144 drd 3	2.6×10^{-1}	2.7×10^{-5}

Hfr P13 cys his X W1655 metB nalA F⁻

* At least 1,000 recombinants were counted per cross in several experiments.

TABLE 12

Extrachromosomal transfer from rec⁻ donor strains

Percentage recipient population converted to donors*

Sex factor	rec⁺ donor	recA donor
F	96	90
F'lac	97	88
Col V2	81	79
Col V3	86	87
Col B1	76	71
Col B2	79	80
Col B3	100	100
Col B4	90	87
Col VB	98	92
R1 drd 19	100	100
R192 drd F7	97	99
Col Ib drd	100	100
R64 drd 11	97	99
R144 drd 3	99	100

HFCT donor cultures of Col B1, Col B2 and Col B4 were used.

metB nalA X thr leu thi proA his argE strA

* Transfer was measured by scoring at least 2,000 infected cells per cross.

(Clowes & Rowley, 1954). Three recombinant classes, *pro*$^+$ *strA*, *his*$^+$ *strA* and *trp*$^+$ *strA* were selected in a 60 minute mating experiment at 37°. The results are shown in Table 13 and are in agreement with the previously obtained data. However, the improved mating conditions have accentuated the deficiencies of the *recA* donors compared to their *rec*$^+$ counterparts.

Again it was concluded that the *recA* mutation does not affect chromosome transfer by either *Col Ib drd* and *R1 drd 19*, although the two plasmids display widely differing transfer frequencies; *Col Ib drd* at a frequency $1.0\text{-}7.0 \times 10^{-9}$ and *R1 drd 19* at a frequency $5.0\text{-}15.0 \times 10^{-3}$ for *trp*$^+$ recombinants. This latter R-factor has been shown to transfer the chromosome from an apparently fixed origin, near the genes for tryptophan biosynthesis (Pearce & Meynell, 1968). The other genetic markers *pro* and *his*, although not transferred as efficiently as the *trp* genes, also show no reduction in transfer from a *recA* donor.

1-D Discussion

Bacteria mutant at *recA* are defective in recombination when used as recipient strains and crossed with Hfr donor bacteria (Clark & Margulies, 1965; Howard-Flanders & Theriot, 1966). The observed reduction in their capacity as recipient strains is not due to an inability to accept genetic material from a donor strain. The transfer of the λ prophage by a lysogenic Hfr occurs normally to *recA* F$^-$ recipients, as judged by the frequency of zygotic induction (Clark & Margulies, 1965). No difficulty was experienced in the infection of these *recA* mutant bacteria with the transmissible plasmids used in these experiments.

TABLE 13

Chromosomal transfer from rec⁻ donors

The frequency of recombinants*

Sex factor	pro⁺A strA		trp strA		his strA	
	rec⁺	recA	rec⁺	recA	rec⁺	recA
F	3.5×10^{-5}	1.1×10^{-8}	1.0×10^{-5}	6.0×10^{-9}	6.0×10^{-6}	3.0×10^{-9}
F'lac	2.7×10^{-2}	2.4×10^{-8}	1.0×10^{-6}	2.0×10^{-9}	4.0×10^{-6}	2.0×10^{-9}
Col V2	4.0×10^{-6}	4.0×10^{-9}	2.4×10^{-6}	5.0×10^{-9}	3.0×10^{-6}	8.0×10^{-9}
Col V3	1.8×10^{-6}	6.1×10^{-9}	3.0×10^{-7}	4.0×10^{-9}	8.0×10^{-7}	4.8×10^{-9}
Col B1	9.1×10^{-7}	6.0×10^{-9}	3.0×10^{-6}	3.8×10^{-9}	4.0×10^{-6}	2.5×10^{-9}
Col B2	7.4×10^{-6}	3.6×10^{-9}	6.0×10^{-6}	4.6×10^{-9}	1.9×10^{-7}	1.8×10^{-9}
Col B3	4.1×10^{-5}	1.4×10^{-8}	2.0×10^{-5}	5.0×10^{-9}	5.0×10^{-6}	5.8×10^{-9}
Col B4	1.8×10^{-6}	7.4×10^{-9}	1.0×10^{-6}	1.2×10^{-8}	4.0×10^{-6}	1.8×10^{-8}
Col VB	4.0×10^{-4}	4.8×10^{-9}	9.8×10^{-1}	9.2×10^{-1}	6.0×10^{-6}	6.0×10^{-9}

Table 13 (Cont'd)

Sex factor	pro⁺A strA		trp strA		his strA	
	rec⁺	recA	rec⁺	recA	rec⁺	recA
R1 drd 19	2.0×10^{-4}	1.8×10^{-4}	7.1×10^{-3}	5.2×10^{-3}	5.0×10^{-6}	5.1×10^{-6}
R192 drd F7	4.0×10^{-6}	4.8×10^{-9}	6.0×10^{-6}	2.0×10^{-9}	1.0×10^{-5}	3.8×10^{-9}
Col Ib drd	5.0×10^{-9}	6.1×10^{-9}	3.8×10^{-9}	4.1×10^{-9}	6.0×10^{-9}	4.8×10^{-9}
R64 drd 11	2.0×10^{-6}	4.2×10^{-9}	4.1×10^{-6}	2.1×10^{-9}	4.2×10^{-6}	2.8×10^{-9}
R144 drd 3	3.0×10^{-7}	5.6×10^{-9}	5.2×10^{-7}	2.4×10^{-9}	6.1×10^{-7}	3.9×10^{-9}

metB nalA X proA his trp strA

HFCT donor cultures of Col B1, Col B2 and Col B4 were used

* Determined by measuring at 500 recombinants per cross in several experiments.

The recombination-deficiency of recA bacteria is accompanied by a concomitant hypersensitivity to both X-irradiation and ultraviolet light (Clark & Margulies, 1965; Howard-Flanders & Theriot, 1966). Although the presence of transmissible plasmids in the recA strains does not restore the recombination ability, the plasmid Col Ib confers considerable protection on them against U.V. damage (Howarth, 1965; Takano, 1966; Walsh & Meynell, 1967), see Experimental Section 3.

All the recA donor bacteria are able to form contacts and transfer their autonomous plasmids to recipient strains, with an efficiency almost equal to the equivalent rec$^+$ donors. Thus the only donor property that is severely affected by the recA mutation is chromosome transfer. This reduction in the ability to sire recombinants is consistent with the idea that the major part of observed chromosome transfer by most transmissible plasmids is dependent upon the bacterial REC system which mediates a recombination event between the plasmid and the chromosome. If the major part of chromosome transfer is REC-dependent, what is the mechanism for the low level of residual transfer from a recA donor strain? Leakiness of the recA mutation is unlikely, since this would lead to a cnnstant rec$^+$/recA ratio of the recombinants produced. The loss of polarity of transfer by an F'lac donor which is recA (Clowes & Moody, 1966) also argues against leakiness of the recA mutation, since a mere diminution in the efficiency of the recA function should not affect the specificity of the integration site of the F'lac factor.

The transfer of chromosome by Col Ib drd and R1 drd 19 is

unaffected by the presence of the recA mutation in the donor strain. If the transfer mediated by these plasmids is by a mechanism which is independent of the bacterial REC system, perhaps the residual transfer by the other recA donors is by the same mechanism. Since Col Ib can mediate the transfer of non-transmissible plasmids such as Col E1 and Col E2 (Smith, Stocker & Ozeki, 1963) without any structural association then, it could perhaps transfer chromosomal material in the same way, providing only a means of making effective contacts with recipient strains. This might then be followed by the passive transfer of chromosome fragments into the recipients(Clowes, 1963a; Clowes & Moody, 1966). In fact, the Col Ib plasmid appears to possess no demonstrable ability to integrate into the chromosome (Edwards & Meynell, 1969; Meynell & Edwards, 1968), see Experimental Section 4. The concept of spontaneous fragmentation of the chromosome, followed by the passive transfer of a fragment into a recipient strain, is not a very satisfactory one. This mechanism would be so inefficient that it would result in a lower frequency of transfer than the observed Col Ib drd level. Such a mechanism almost certainly could never attain the efficient transfer of chromosome mediated by R1 drd 19, which is also apparently independent of the REC system.

It seems more likely that these two plasmids, and possibly all transmissible plasmids, can themselves specify an interaction with the bacterial chromosome, the efficiency and frequency of this interaction being dependent on the particular plasmid and chromosome involved. Perhaps, where there is a long period of evolutionary

association between the plasmid and its bacterial host, as in the case of F, the recombination systems of both genomes may co-operate to give stability to complexes between them, the most stable event being true integration by a reciprocal genetic exchange. This apparently is a rare occurrence with transmissible plasmids other than the F sex factor of E. coli since, with the exception of the Hfr donors isolated from Col V2 donor strain (Kahn, 1968), it has been impossible to detect Hfr-type donors in the case of the other plasmids studied, except by very special techniques (see Experimental Section 4). However, even with highly selective methods no Hfr type donors have been isolated in donor strains harbouring Col Ib or any I-like plasmids. It is proposed that these plasmids can specify their own interaction with the chromosome but are unable to participate in a reciprocal recombination event.

Appendix 2

See attached publication

Chromosomal transfer from "Recombination-deficient" strains of Escherichia coli K-12

R. C. Clowes & E. E. M. Moody

Genetics 53, 4 (1966), 717-726.

The initial observations reported here have been extended. The development of new donor strain and the modification of the experimental protocol have increased the scope of the study. These extensions of the research are reported in Experimental Section 1.

Reprint from GENETICS, Vol. 53, No. 4, April, 1966. *Printed in U.S.A.*

CHROMOSOMAL TRANSFER FROM "RECOMBINATION-DEFICIENT" STRAINS OF *ESCHERICHIA COLI* K-12

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Received November 29, 1965

CERTAIN strains of Enterobacteria harbor infectious genetic elements known as sex factors (HAYES 1964) or transfer factors (GROSS 1965) which enable them to act as genetic donors. That is, cells of such donor strains are able to form stable unions with other cells and transfer to them extrachromosomal material (which may include the sex factor itself) and, more rarely, segments of bacterial chromosome.

The first discovered (HAYES 1953; CAVALLI, LEDERBERG and LEDERBERG 1953) and best documented (JACOB and WOLLMAN 1961) of these factors is the F fertility factor of *Escherichia coli* K-12. Since that time other genetic elements have been found which control other cellular properties in addition to sex factor activities. Among these elements are the *colicin factors* (OZEKI and HOWARTH 1961; CLOWES 1961) and the multiple *drug-resistance transfer factors* (SUGINO and HIROTA 1962).

The F sex factor, together with the temperate bacteriophage λ, formed the model on which the "episome" concept of JACOB and WOLLMAN (1958) was based; an episome being defined as a nonessential genetic element which could exist either autonomously or integrated with the chromosome. Although in the wild state, the F sex factor is an autonomous, extrachromosomal element (in F^+ donors), stable Hfr strains can be isolated from cultures of F^+ strains, which transfer their chromosome with high frequency and in an oriented manner. In each Hfr strain, the F sex factor is suggested to be integrated at one of a number of alternative sites on the K-12 chromosome by a mechanism involving pairing between the F factor and chromosome, followed by a reciprocal genetic exchange (ADELBERG and PITTARD 1965; GROSS 1965). It has moreover been suggested that the entire fertility of F^+ strains may reside in the small number of Hfr mutants present in every large F^+ population.

It was at one time concluded (ALFOLDI, JACOB, WOLLMAN and MAZÉ 1958) that certain colicin factors also occupy a chromosomal site and thus qualify as episomes, but the conclusions of these experiments have been questioned (CLOWES 1963b) and a model of colicin factors as stable, autonomous plasmids has been proposed (MONK and CLOWES 1964b). Nevertheless, it could still be suggested that occasional integration of a colicin sex factor with the chromosome might

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occur which would account for its ability to mediate the transfer of chromosomal markers.

More recently, "recombination-deficient" (*rec*⁻) mutants of K-12 have been isolated by CLARK and MARGULIES (1965) which are suggested to be unable to catalyze one or more steps involved in the recombination process. The extent to which these *rec*⁻ mutants can act as genetic donors when infected with one of

TABLE 1

Bacterial strains (all strains are E. coli *K-12 sub lines except where noted)*

Strain	Genotype: Chromosome	Genotype: Sex factor	Origin or derivation
(a) *Sources of sex factors*			
501	(58–161) *met*	F	Original strain (HAYES 1952)
770	(W1655) *met lac*+	F-*lac*+	SCAIFE and GROSS (1962)
519	(58–161) *met*	colI	MONK and CLOWES (1964a)
414	(*E. coli* K94) prot	colV2	Original strain K94 (FRÉDÉRICQ, 1963) see MACFARREN and CLOWES (1966)
810	(J62) *his try thy str*^{r}	colV3	MACFARREN and CLOWES (1966)
(b) *Strains derived from AB1157 (thr leu thi pro his arg lac*⁻*xyl*-*T6*^{r} *str*^{r}*)*			
(i) "Recombination-active" (rec+) *strains*			
97	*rec*+	0(F⁻)	Original strain (ADELBERG 1962)
112	*rec*+	F	97 × 501
101	*rec*+	F-*lac*+	97 × 770
100	*rec*+ *colI*^{r}	0(F⁻)	97 × colicin I
108	*rec*+ *colI*^{r}	colI	100 × 519
110	*rec*+	colV2	97 × 414
131	*rec*+	colV3	97 × 810
(ii) "Recombination-deficient" (rec⁻) *strains*			
96	*rec*⁻	0(F⁻)	HOWARD-FLANDERS and THERIOT (AB 2463)
111	*rec*⁻	F	96 × 501
107	*rec*⁻	F-*lac*+	96 × 770
99	*rec*⁻ *colI*^{r}	0(F⁻)	96 × colicin I
106	*rec*⁻ *colI*^{r}	colI	99 × 519
109	*rec*⁻	colV2	96 × 414
130	*rec*⁻	colV3	96 × 810
(c) *Other strains*			
902	*(Salmonella typhimurium)* LT-2 *cys36*	colI	OZEKI and HOWARTH (1961)
750	Hfr C (*met*)	F	Original strain (CAVALLI 1950)
C600	*thr leu thi lac*⁻	0(F⁻)	Original strain (APPLEYARD 1954)
502	(58–161) *met str*^{r}	0(F⁻)	Original strain (HAYES 1952)
510	(58–161) *met str*^{r} *colI*^{r}	0(F⁻)	502 × colicin I

prot indicates growth on minimal medium without amino acid or vitamin supplements. *met, thr, leu, thi, pro, his, arg, cys, thy:* requirement for methionine, threonine, leucine, thiamine, proline, histidine, arginine, cysteine, or thymine. *str*^{s} (*str*^{r}): sensitivity (resistance) to streptomycin (200 μg/ml). *lac*$^+$ *xyl*$^+$ ability to ferment the sugars lactose or xylose; *lac*⁻, *xyl*⁻: inability to ferment these sugars. Strain 96 is a mutant isolated from strain 97 by P. HOWARD-FLANDERS which is hypersensitive to ultraviolet and also to X-irradiation. F is the F fertility factor of K-12; colI, colV2, colV3 are colicin factors which determine the ability of a strain to produce colicin I or colicin V. colI^r indicates strains selected resistant to colicin I and which are also cross-resistant to colicin V.

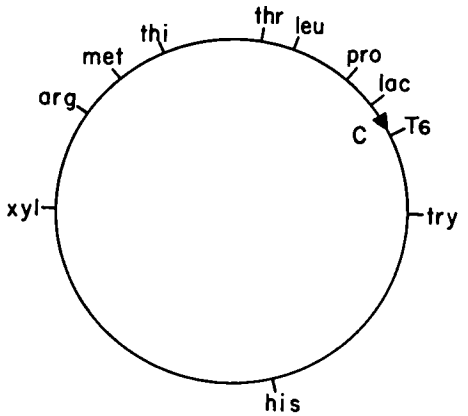

FIGURE 1.—Disposition of markers on the circular chromosome of K-12. "C" represents orientation of transfer of Hfr Cavalli.

several alternative sex factors might be expected to clarify whether recombination between a sex factor and the chromosome is a necessary prerequisite for chromosomal transfer. This would, of course, require that infection by sex factors does not promote the formation of recombinants in the *rec*⁻ strain. This paper reports an investigation of these properties using a mutant of the *rec*⁻ type (HOWARD-FLANDERS and THERIOT 1966) infected with F, or with one of several distinct colicin factors.

MATERIALS AND METHODS

Bacterial strains: The strains used in this study are listed in Table 1.

Media: All media used have been described in a previous publication (MONK and CLOWES 1964a).

Techniques of infection with F, and with colicin factors, of F testing and of colicinogeny testing are described elsewhere (MONK and CLOWES 1964a). Standard crossing techniques were used in bacterial matings (see CLOWES 1961; MONK and CLOWES 1964a; MACFARREN and CLOWES 1966).

RESULTS

Efficiency of rec⁻ *strains as recipients:* The first experiments tested the ability of the parental *rec*⁻ strain to act as a recipient when mated with the Hfr donor, Hfr CAVALLI. When one of a number of sex factors had been introduced into this *rec*⁻ strain, its capacity as a recipient was similarly investigated with the results shown in Table 2. It can be seen that in contrast to the control crosses using *rec*⁺ recipients, where transfer of a proximal marker, *pro*⁺, led to about 5% recombinants, the frequency of *pro*⁺ recombinants issuing from *rec*⁻ recipients was reduced by a factor of at least 1 in 5000. Moreover, when the factors F or F-*lac*⁺ were present in the recipient, the recombinants were reduced even further, as would be expected from crosses between two donor strains (see HAYES 1964).

Nonchromosomal transfer from rec⁻ *donors:* The extent to which *rec*⁻ strains, when infected with one of the several sex factors, can conjugate and transfer

TABLE 2

Efficiency of rec⁻ *strains as recipients*

Donor × Recipient

Hfr Cavalli × AB 1157

Recipient	Mean number *pro*⁺ colonies per plate	Total count Hfr ($\times 10^8$)	Overall dilution	Frequency *pro*⁺ recombinants per input Hfr
rec⁺ F⁻	304	3	10^{-3}	5×10^{-2}
colI⁺	298	2.4	10^{-3}	6.2×10^{-2}
colV3⁺	358	3	10^{-3}	6×10^{-2}
rec⁻ F⁻	623	3	$\times 10$	1×10^{-5}
F⁻	592	2	$\times 10$	1.5×10^{-5}
F⁺	17/5	2	$\times 10$	8.5×10^{-8}
F-*lac*⁺	13/5	2	$\times 10$	6.5×10^{-8}
colI⁺	538	2.4	$\times 10$	1.1×10^{-5}
colI⁺	135	2	$\times 10$	3.4×10^{-6}
colV3⁺	601	3	$\times 10$	1×10^{-5}

1 ml young (ca. 2×10^8) Hfr C culture mixed with 9 ml (0/N dil. 1/10 = ca. 2×10^8) AB1157 recipient culture was incubated for 2 hr at 37° unshaken, washed twice in 10 ml buffer, resuspended in 1 ml ($\times 10$) and 0.2 ml plated in quintuplicate either direct (*rec*⁻ crosses) or after 10^{-4} dilution (*rec*⁺ controls), on minimal medium supplemented with threonine, leucine, thiamine, histidine, arginine and streptomycin (to select *pro*⁺ recombinants).

their sex factor to the same normal (*rec*⁺) recipient strain (C600 F⁻) was next investigated and compared with the transfer from the corresponding *rec*⁺ donor strains. The results shown in Table 3 show that although transfer of either F or F-*lac*⁺ from *rec*⁻ donors is reduced, it still remains at a value greater than a half that from *rec*⁺ donors. Transfer of the three colicin factors however was almost equally efficient from either *rec*⁻ or *rec*⁺ strains. In the case of the colI factor, transfer was similar whether measured at the low level found from stably colicinogenic cells (low frequency colicinogeny transfer-LFC) or from a strain newly infected with colI from which transfer is enhanced (high frequency colicinogeny transfer-HFC; see STOCKER, SMITH and OZEKI 1963; MONK and CLOWES 1964a).

Chromosomal transfer from rec⁻ *donors:* The transfer of chromosomal markers from these *rec*⁻ strains, each infected with one of the several sex factors, to the same common recipient strain, was next investigated. These experiments, together with the control crosses using the corresponding *rec*⁺ donors, are shown in Table 4. The multiply-deficient auxotrophic genotype (*thr leu thi pro his arg*) of the *rec*⁺ and *rec*⁻ parental strains, limited the number of strains with suitable markers that could be used as recipients, and the strain chosen carried a *met* mutation. Selection for *met*⁺ recombinants was therefore made on minimal agar supplemented with arginine and thiamine. This avoided counter selection of the recipient markers *arg*⁺ and *thi*⁺, which are closely linked to either side of *met*⁻ and would otherwise have reduced the overall yield of recombinants.

Several features can be seen in the data of Table 4. First, with the notable exception of colI-mediated transfer, the numbers of *met*⁺ recombinants produced by *rec*⁻ donors were much inferior to those derived from the corresponding *rec*⁺ donors, ratios ranging from only 0.13% of the *rec*⁺ level in F-*lac*⁺ crosses to

TABLE 3

Nonchromosomal transfer from rec⁻ *donors*

Donor × Recipient

AB 1157 (*xyl*-*lac*⁻) C600 (*xyl*+*lac*⁻) F⁻

Donor			Sex factor transfer		
			μ^s		
F+		*rec*+	37/40	(93%)	
		rec⁻	22/40	(56%)	
			μ^s *lac*+	μ^s *lac*⁻	μ^r *lac*+
F-*lac*+		*rec*+	39/40 (98%)	0/40	0/40
		rec⁻	21/40 (54%)	0/40	0/40
			colI+		
colI+	LFC	*rec*+	4/48 (8%)		
	LFC	*rec*⁻	4/48 (8%)		
	HFC	*rec*+	46/50 (92%)		
	HFC	*rec*⁻	45/50 (90%)		
			μ^scolV2+	μ^rcolV2+	μ^scolV2⁻
colV2+		*rec*+	30/48 (63%)	0/48	0/48
		rec⁻	26/48 (54%)	0/48	0/48
			μ^scolV3+	μ^rcolV3+	μ^scolV3⁻
colV3+		*rec*+	48/48 (100%)	0/48	0/48
		rec⁻	47/48 (98%)	0/48	0/0

Equal volumes young cultures (ca. 2×10^8) of both donor and recipient strains were mixed for 2 hr at 37°* and then diluted and plated on EMB xylose for single colonies. A number of well isolated *xyl*+ colonies were then purified by streaking and tested for transfer of the sex factor concerned: F in F+ and F-*lac*+ strains by sensitivity (μ^s) to the male specific phage, μ_2, colI by overlay with C600F⁻ as a colicin I-sensitive strain; and colV2 and colV3 by both overlay with the colicin V sensitive strain (C600F⁻) and also by sensitivity of each colony to the male specific phage, μ_2. (All strains carrying colV2 and colV3 are also sensitive to male-specific phage, μ, – see MACFARREN and CLOWES 1966). A proportion of the μ^s and μ^r colonies were tested for fertility. 20/20 μ^s colonies from F-*lac*+ crosses were fertile; 0/20 μ^r colonies were fertile. 12/12 μ^s colonies from colV2 crosses were fertile; 0/5 μ^r colonies were fertile. 8/8 μ^s colonies from colV3 crosses were fertile, one μ^r colony was infertile.

* 200 μg/ml trypsin was added to cultures to prevent lethal effects of free colicins on the recipient.

10.1% in the colV2 cross. Secondly, irrespective of the frequency of *met*+ recombinants produced by these various *rec*+ donors (from 1.5×10^{-5} in the case of F-*lac* to 8×10^{-8} with colV3), the frequency from the corresponding *rec*⁻ donors was very similar (within the range 0.61 to 2.1 $\times 10^{-8}$). Finally, this low level of transfer from *rec*⁻ donors was found in *all* crosses involving sex factors, and was at least 200-fold the maximum possible level in mixtures when *no* sex factor was present (whether *rec*+ or *rec*⁻ mixtures) and was in fact very similar to the level of transfer from colI donors in the HFC state, whether *rec*+ or *rec*⁻.

The relative frequencies with which the two unselected markers, *xyl*⁻ and *T6*^{r} lying on opposite sides of the circular K-12 chromosome (see Figure 1) appear in *met*+ recombinants in these various crosses is shown in Table 5. It has been previously demonstrated that there is no obvious preference for the transfer of markers from any one segment of the chromosome, in crosses from normal *rec*+ strains mediated either by *autonomous* F (CLOWES and ROWLEY 1954; CAVALLI-SFORZA and JINKS 1956) by ColI (CLOWES 1961) or by colV2 (MACFARREN and CLOWES 1966). In contrast, the transfer due to an F prime factor such as F-*lac* is biased owing to the preferred integration of the factor at the site of the chromosomal fragment which was previously incorporated as part of the F-prime struc-

TABLE 4

Chromosomal transfer from rec⁻ *donors*

Donor AB 1157 (*thr leu thi pro his arg T6ʳ xyl⁻*) × Recipient* 58–161 (*met T6ˢ xyl⁺*) *F⁻*

Donor			Experiment number	Total cells plated ($\times 10^9$)	*met⁺arg±thi±* colonies	Rec. freq. ($\times 10^{-8}$)		Efficiency *rec⁻/rec⁺*
F+		*rec*+	1	4	1853	46	44	
			3	10	4078	41		2.7%
		rec⁻	1	4	54	1.3	1.2	
			3	10	109	1.1		
F-*lac*+		*rec*+	5	10	157,400	1574		0.13%
		rec⁻	1	4	127	3.2	2.1	
			5	10	103	1.0		
colI+	LFC	*rec*+	1	4	11	0.28	0.25	
			2	10	24	0.24		0.92%
			3	10	23	0.23		
	LFC	*rec*⁻	1	4	9	0.22	0.23	
			2	10	22	0.22		
			3	10	28	0.28		
	HFC	*rec*+	6	10	182	1.82	1.80	0.69%
			8	10	177	1.77		
	HFC	*rec*⁻	6	10	86	0.86	1.24	
			8	10	161	1.61		
colV2+		*rec*+	4	6	1567	26.2	16.8	
			7	10	1121	11.2		10.1%
		rec⁻	4	6	168	2.8	1.7	
			7	10	101	1.0		
colV3+		*rec*+	7	20	1688	8.4		7.3%
		rec⁻	7	20	122	0.61		
F⁻ (control)		*rec*+	4	10	0	< .003		
			7	20	0			
		rec⁻	4	10	0			
			7	10	0			

50 ml young cultures (ca. 2×10^8) of both donor and recipient strains are mixed at 37° for 2 hr (static), washed twice in 100 ml buffer and resuspended in 5 ml ($\times 20$). Volumes of 0.2 ml are then plated on minimal medium supplemented with arginine, thiamine, and streptomycin and incubated 48 hr at 37°.

* Resistant to colicin I (*colIʳ*) and cross resistant to colicin V2 and V3.

TABLE 5

Unselected markers among recombinants

Donor AB 1157 (*thr leu thi pro his arg xyl*⁻ *T6*ʳ) × Recipient 58–161 (*met xyl*⁺ *T6*ˢ) *F*⁻

met⁺ recombinants with donor marker*	Donor									
	F-lac⁺		F⁺		colI⁺		colV2⁺		colV3⁺	
	rec⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻	*rec*⁺	*rec*⁻
xyl⁻	10/48 (21%)	9/48 (19%)	17/63 (27%)	9/63 (14%)	6/63 (10%)	9/63 (14%)	24/80 (30%)	33/128 (26%)	16/90 (18%)	22/90 (24%)
*T6*ʳ	1/366 (0.25%)	6/103 (6%)	5/63 (8%)	3/63 (5%)	8/63 (12%)	6/63 (10%)	6/80 (8%)	7/128 (6%)	10/90 (11%)	13/90 (14%)

* Pooled recombinants from crosses shown in Table 4 were tested for these unseleced markers.

ture, in this case *lac*, which lies between *pro* and *T6*. This particular F-prime transfers *pro*⁺ as an early (proximal) marker and *T6* as a late (distal) marker (SCAIFE and GROSS 1962). The results from *rec*⁺ donors shown in Table 5 are in line with these observations, the donor *xyl* marker being found in between 10 and 30% of the *met*⁺ recombinants in all crosses, whereas although the *T6* marker is found in between 8 and 12% recombinants from F, colI, colV2 and colV3 mediated crosses, it is found in only 0.25% of the recombinants from F-*lac* donors.

In contrast, the incorporation of both markers is more nearly the same from all *rec*⁻ donors, including those from F-*lac rec*⁻ donors; *xyl* appearing in 14 to 26% of the recombinants and *T6* in 5 to 14%.

DISCUSSION

The results of Table 2 show that the *rec*⁻ strain 96 (AB2463 of HOWARD-FLANDERS and THERIOT) is defective *when used as a recipient.* It has been assumed that this defect is probably due to the inability to integrate the genetic material which is received from the donor, as has been concluded in the case of the similar strain isolated by CLARK and MARGULIES (1965), and it is *not* due to the lack of ability to accept this genetic material from the donor. This latter aspect is supported by the finding that no difficulty was experienced in infecting AB 2463 with the various sex factors used in this study. Moreover, this strain has been shown by HOWARD-FLANDERS and THERIOT to be hypersensitive to both ultraviolet and X-irradiation to the same extent as the strain of CLARK and MARGULIES. It may also be concluded from Table 2 that infection of this *rec*⁻ strain with any of the sex factors used in this study does not compensate this defect, and that if this is due to lack of recombination, then recombination is still not possible in the *rec*⁻ donor strains.

When used as donors, all the *rec*⁻ strains infected with various sex factors were able to form contacts and transfer their sex factor with near-normal efficiency to the standard recipient C600F⁻ strain (Table 3). This suggests that the donor

properties including the formation of stable unions and the transfer of non-chromosomal elements are not greatly affected by the *rec*⁻ mutation. The production of recombinants, and by inference, chromosomal transfer is, on the other hand, markedly reduced from all donor strains carrying the *rec*⁻ mutation, with the exception of those carrying colI (Table 4). This is consistent with the idea that the major part of chromosome transfer mediated either by F (or F-*lac*), colV2 or colV3 depends on the recombination of the sex factor and the chromosome (as has also been concluded in the case of F or F-*lac* by ADELBERG and PITTARD (1965) and by SCAIFE and GROSS (1964)) and this recombination is precluded by the *rec*⁻ mutation.

The results from the study of unselected markers (Table 5) support this main conclusion. Here it is seen that transfer from an F-*lac rec*⁻ donor does not show the bias against a distal marker such as *T6*, as is shown by the F-*lac rec*⁺ donor. It can be concluded that transfer from the *rec*⁺ donor arising from recombination of the F-prime element with the preferred (*lac*) site, is no longer possible, and that the greatly reduced and nonpolarized transfer from the F-*lac rec*⁻ donor is due, as is that from all *rec*⁻ donors, to events which do not involve recombination of sex factor and chromosome. These results would also seem to eliminate a transfer mechanism involving a transient, unstable structural association of sex factor and chromosome, not necessarily involving recombination, since this would also be likely to show a bias for the preferred site in F-prime transfer. The finding of a constant level of recombinants from all *rec*⁻ donors, irrespective of the differences shown by the corresponding *rec*⁺ donors, supports the idea of a low level transfer from *all* donors, which is independent of interaction of sex factor and chromosome. "Leakiness" in the *rec*⁻ mutation, on the other hand, would be expected to lead to a constant *rec*⁺/*rec*⁻ ratio of recombination.

In the case of colI, it appears that the low level of chromosomal transfer normally seen (from colI⁺ *rec*⁺ donors) is not due in any extent to recombination of sex factor and chromosome. This is supported by the fact that this low level transfer, shown by colI donors in the HFC state, whether in *rec*⁺ or *rec*⁻ strains, occurs at a similar level to that which is shown by all other *rec*⁻ donors. (The level shown by colI in the LFC state is further reduced due to the lower frequency with which this factor exhibits all sex-factor properties, including formation of unions and transfer of extrachromosomal elements, when stabilized in a cell [see Table 3 and MONK and CLOWES 1964a]).

Chromosome transfer from colI donors therefore seems at all times to be independent of interaction of sex factor and chromosome. This is a situation parallel to that involving the transfer of other noninfective extrachromosomal elements such as colE1 and colE2 that can be brought about by sex factors like F or colI. In these cases, there is also apparently no structural association between the mediating and the passive extrachromosomal elements (e.g. transfer of colE1 or colE2 by colI in Salmonella (SMITH, OZEKI and STOCKER 1963) or of colE1 and colE2 by F (CLOWES 1964). Chromosome transfer by colI might depend only upon spontaneous fragmentation of the normally circular K-12 chromosome to form a linear structure, as has been suggested in an earlier publication (CLOWES

1963a), and the colI factor seems to have no demonstrable ability for association or integration with the chromosome as have the other sex factors used in this study.

We are indebted to DR. P. HOWARD-FLANDERS for the *rec*+ and *rec*⁻ parental strains and for unpublished information.

SUMMARY

Chromosome transfer mediated by certain sex factors (F and F-primes, colV2 and colV3) is greatly reduced from donor strains which have a "recombination-deficient" (*rec*⁻) mutation, when compared to transfer from the corresponding normal (*rec*+) donors. It is concluded that the major part of chromosome transfer mediated by these factors is dependent upon recombination of sex factor and chromosome. Nevertheless, chromosomal transfer *is* in fact found in all cases at a decreased level which is fairly constant in crosses involving any of these sex factors. Unselected marker transfer supports the idea that this low level transfer does not depend upon recombination or on a transient association of sex factor and chromosome. Chromosome transfer by another factor such as colI is similar whether from *rec*+ or *rec*⁻ donors from which it is concluded that such factors may be incapable of structural association or integration with the bacterial chromosome.

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CHAPTER 4

THE STIMULATION OF CHROMOSOME TRANSFER

Shortly after the discovery of recombination in E. coli K12, it was reported that irradiation of the parents of a cross with ultraviolet light stimulates the formation of recombinants (Haas, Wyss & Stone, 1948). With the discovery of sexual differentiation Hayes (1952a) demonstrated that the stimulation of recombinant formation is due entirely to an increased fertility of the F^+ donor population. If the F^- recipient bacteria are irradiated, the numbers of recombinants fall proportionately with the U.V. survival rate of the F^- population. This is not surprising since the F^- strain is the parent which constitutes the zygote wherein the entire recombination and segregation processes occur. Similarly, the fertility of Hfr donor strains falls with increasing U.V. damage, paralleling the viable count.

The U.V. stimulation of recombinant formation was shown to be photo-reactivable and to require incubation of the irradiated F^+ donor in broth for its development (Hayes, 1953b). The stimulation of fertility by U.V. irradiation can produce as much as a 50-fold increase in the total number of recombinants under optimal conditions. The increased donor ability to generate recombinants is not inherited by the descendents of the induced F^+ donor bacteria (Hayes, 1960) and there is no appreciable increase in the number of stable Hfr donors formed.

The phenomenon has been redefined as the induction, by U.V. irradiation, of new chromosomal donors in bacterial populations

which harbour autonomous sex factors; this induction is reflected in an increase in the absolute number of recombinants generated (Evenchik, Stacey & Hayes, 1969).

The induction of new chromosomal donors also occurs following treatment with mitomycin C, thymine starvation or nitrous acid, but does not develop after X-irradiation, treatment with hydroxylamine, caffeine, or methyl methane sulphonate (MMS), or following the incorporation of 5-bromouracil (Evenchik et al, 1969; Rajchert-Trzpil & Dobrzański, 1968).

The damage produced in DNA by U.V. irradiation and the inhibition of DNA synthesis is known to activate the enzymatic repair mechanisms of the bacterial cell (Boyce & Howard-Flanders, 1964a; Howard-Flanders, Boyce & Theriot, 1966), which probably also operates on DNA damaged by mitomycin C (Boyce & Howard-Flanders, 1964b). The stimulating effects of these mutagenic agents on the fertility of $\underline{F}^+$ donor strains could, therefore, perhaps be the activation of DNA repair synthesis. The repair processes might favour the recombination event between the sex factor and the chromosome (Rajchert-Trzpil & Dobrzański, 1968). These authors claim that caffeine treatment of U.V. irradiated donor bacteria, before conjugation, enhances the effect of the U.V. stimulation. Caffeine is believed to inhibit U.V. dark repair (Lieb, 1961; Metzger, 1964).

The agents that produce the U.V. stimulation effect all yield the type of DNA damage which is repaired by an excision process. The damaged single strand fragments of DNA, containing pyrimidine dimers are excised and acid soluble nucleotides appear in the medium

(Boyce, 1966; Boyce & Howard-Flanders, 1964a; Setlow & Carrier, 1964). The result of this excision is that single stranded gaps are exposed in the DNA. These gaps are repaired by new DNA synthesis using the interrupted strand as a primer and the intact, complementary strand as a template (Pettijohn & Hanawalt, 1964). When the $\underline{F}^+$ donor is defective in the excision of pyrimidine dimers from its DNA after U.V. irradiation ($\underline{uvr}^-$) (van de Putte, van Sluis, van Dillewijn & Rörsch, 1965) or is unable to carry out normal recombination (rec^-) the U.V. stimulation of fertility does not take place (Evenchik et al, 1969). Evenchik et al therefore suggested that the excision of a U.V. damaged strand of chromosomal DNA in a region of sex factor homology could lead to facilitation of pairing of the sex factor with the intact chromosomal strand. This would be followed by breakage of the sex factor strand and its covalent bonding to the free end of the excised strand, by means of the bacterial REC system. This unorthodox structure, in which sex factor and chromosome are joined by only a single DNA strand, has no obvious barrier to a replication which is initiated in the sex factor, continuing along the chromosome to effect chromosome transfer. However, a second replication of the chromosome, or the completion of a single replication initiated at the chromosomal origin, will be blocked at the sex factor attachment site. Thus, this donor would be inviable.

In support of this model, the U.V. stimulation is inhibited in irradiated $\underline{uvr}^+\underline{F}^+$ donors which have been treated with acriflavine,

a dye reported to prevent the excision of pyrimidine dimers (Harm, 1967; Setlow, 1966). Moreover, Hayes (1953) demonstrated that the effect is reversed by photo reactivation, which is known to specifically uncouple pyrimidine dimers (Rupert, 1961; Setlow, Carrier & Bollum, 1965).

Experimental Section 2

The effect of U.V. irradiation on the fertility of donor strains, carrying many different transmissible plasmids was investigated. Does the U.V. stimulation of chromosome transfer in all these sexual systems? The donor strains used in this study are listed in Table 6. The recipient strains were J62 proA his trp strA F^- and PA309 thr leu thi trp his argH strA F^-.

2-A The ultraviolet stimulation of chromosome transfer

Aliquots of all the donor cultures were irradiated in phosphate buffer to a survival of c.30%. Immediately after irradiation the cultures were transferred to screw-capped bottles and $^1/_{20}$th volume of X20 nutrient broth added. The bottles were wrapped in silver foil, to prevent photoreactivation and incubated on an inclined turntable. The unirradiated control cultures were maintained in log phase, without multiplication during this time. Both donor preparations were then mated with the same recipient under identical conditions. The effect of U.V. irradiation on the fertility of a series of isogenic donor strains is shown in Table 14. All irradiated donor strains show some increase in the total number of recombinants, for at least one of the recombinant classes examined. The colicinogenic factors Col B1, Col B2 and Col B4 are fertility-repressed

TABLE 14

A comparison of the effect of U.V. irradiation on the fertility of isogenic donor strains

The total number of recombinants/ml.

Sex factor	Before U.V.	After U.V.		Increase
F	8.0×10^3	2.6×10^5	*a.e	X 32
F'lac	7.0×10^5	2.8×10^6	a	X 4
Col V2	6.0×10^2	1.0×10^4	b	X 17
Col V3	4.8×10^2	9.1×10^4	c	X 19
Col B3	4.9×10^3	1.7×10^5	a.b.e	X 34
Col VB	8.8×10^3	4.4×10^4	a	X 5
R1 drd 19	2.4×10^3	7.2×10^3	c	X 3
R192 drd F7	8.8×10^2	1.4×10^4	d	X 16
Col Ib drd	1.0	7.9×10^1	b.c.	X 79
R64 drd 11	4.0×10^2	1.9×10^4	c	X 47
R144 drd 3	6.0×10^2	3.2×10^4	e	X 53

* recombinants class selected a. $\underline{pro^+A}$

b. $\underline{arg^+H}$

c. $\underline{thr^+}$ $\underline{leu^+}$

d. $\underline{trp^+}$

e. $\underline{his^+}$

At least 500 recombinants were scored per cross in several experiments.

plasmids. To measure the effect of U.V. irradiation on their fertility it was necessary to prepare HFCT donor cultures. After overnight growth, followed by sub-culture and a further $2\frac{1}{2}$ hr. incubation, these newly infected intermediate donors were irradiated. The infection of the intermediate donors was always better than 95%. The effects of irradiation on chromosome transfer by these HFCT cultures is shown in Table 15. The ultraviolet irradiation stimulates both LFCT and HFCT donor cultures. The increased number of recombinants sired by the LFCT cultures must reflect, the degree of derepression of the conjugal functions produced by U.V. irradiation, since both irradiation and the preparation of HFCT donor cultures produce the same increased frequency of recombinants (see Table 15). However, it is probable that, in both cases, part of this increase is due to a superimposed enhancement of chromosome transfer.

Those plasmids which have incorporated parts of the bacterial chromosome into the plasmid genome are the relatively least stimulated by U.V. irradiation, although the total number of recombinants produced are, of course, much greater. In fact, the total number of recombinants yielded by these irradiated donors approximates the number expected from an Hfr donor. It is therefore not surprising that the F'lac, Col VB trp cys and R1 drd 19 factors do not show a large increase in the number of recombinants. These three plasmids already interact very efficiently with the bacterial chromosome. There is no evidence that R1 drd 19 has any chromosomal material as part of its genome, but it does transfer the

TABLE 15

A comparison of the effect of the U.V. irradiation on the fertility of LFCT and HFCT cultures of Col B^+ donors

The total number of recombinants/ml

Sex factor	Before U.V. LFCT	After U.V. LFCT	Increase	Before U.V. HFCT	After U.V. HFCT	Increase
Col B1	1.8×10^1	9.0×10^1	X 5	2.4×10^2	*b.c. 4.6×10^3	X 19
Col B2	1.6×10^1	1.3×10^2	X 8	1.4×10^2	*d.e. 2.2×10^3	X 16
Col B4	8.0×10^1	7.2×10^2	X 9	7.2×10^2	*a.c. 1.2×10^4	X 16

* recombinant classes selected a. pro^+A

b. arg^+H

c. $thr^+ leu^+$

d. trp^+

e. his^+

At least 500 recombinants were scored per cross in several experiments.

chromosome at a very high frequency from a fixed origin which is near the genes for tryptophan biosynthesis (Pearce & Meynell, 1968). The greatest stimulation of recombinant formation by U.V. irradiation was found with a fertility-derepressed mutant of Col Ib. In some experiments there was more than a 100-fold increase in the total yield of recombinants.

2-B Discussion

The observations of the effect of ultraviolet irradiation on the fertility of donor strains (Hayes, 1952a, 1953b; Rajchert-Trzpil & Dobrzański, 1968; Evenchik et al, 1969) have been confirmed and extended to a wide range of plasmids. U.V. irradiation is capable of stimulating chromosome transfer in all these plasmid conjugation systems. However, it seems doubtful that the model proposed by Evenchik et al (1969) can provide a full explanation of the data obtained with Col Ib drd. In conditions where the Col factor transfer to a recipient,population is over 100% due to epidemic spread, the frequency of recombinants for chromosomal genes is as low as 10^{-8}-10^{-9}. If one assumes that this indicates an exceptionally low degree of homology between the plasmid and the chromosome, it is surprising that U.V. irradiation of a Col Ib drd donor can cause as much as a 100-fold increase in total number of recombinants the highest degree of stimulation observed for any plasmid. If the sole effect of irradiation were due to the excision of U.V. damage to expose single stranded regions of DNA, thus increasing the efficiency of pairing, one would expect that the

lower the inherent homology, the lower would be the extent of enhancement of pairing and the smaller the relative increase in the yield of recombinants. It seems far more likely that a major effect of the ultraviolet irradiation, in this case at least, is to induce a hitherto repressed plasmid function, which is able to promote an interaction with the bacterial chromosome.

Experimental Section 3

The irradiation of repressed donor bacteria with ultraviolet light produces an increase in the efficiency of conjugal plasmid transfer similar to that obtained by the preparation of an HFCT donor culture. This was attributed to the destruction of a repressor by U.V. and the absence of repressor in the newly infected bacteria (Monk & Clowes, 1964b). In view of the stimulation of chromosome transfer produced by U.V. irradiation of donor strains, it seemed important to see if the process of new infection also stimulated chromosome transfer by these transmissible plasmids.

3-A The stimulation of chromosome transfer following new infection with transmissible plasmids

It is essential in this study that only the transfer of chromosomal markers from the intermediate donors, and not from the original donor bacteria, is measured. To ensure this, selection was made for a recombinant class to which the original donor strain could not have contributed. The genotypes of the primary donors and secondary donor strains are shown in Table 16. Since the primary donor and recipient strains have identical auxotrophic requirements, the only possible recombinants must be sired by the secondary intermediate

TABLE 16

The strains used to demonstrate the stimulation of chromosome transfer in newly infected donor bacteria

The primary donor strains were W1655 *nalA* F^- derivatives infected with the plasmids listed in Table 5.

The secondary donor strain used in all the crosses was JC5455 *his trp spcA* F^- Achtman, Willetts & Clark (1971).

The recipient strain used throughout these experiments was a streptomycin resistant isolate of W1655F^-, derived for these experiments. W1655 *strA* F^-

The new infection of secondary donors was achieved as follows:

Primary donor		Secondary donor		Recipient
metB *nalA*	X	*his trp spcA* F^-	X	*metB strA* F^-

Where HFCT donor cultures were required, new infection of the secondary donors was obtained in the following way.

Primary donor (HFCT culture)		Secondary donor		Recipient
(*metB nalA* X *metB* F^-)	X	*his trp spcA* F^-	X	*metB strA* F^-

metB$^+$ *strA* recombinants can only arise from transfer from the secondary donor strains.

donor strain. In the case of repressed plasmids, the primary donor must of course be an HFCT preparation, so that three strains are used successively as donors in the cross, as shown in Table 16. The effects of new infection on chromosome transfer by various plasmids is shown in Tables 17 & 18. The results are similar to those obtained in the U.V. stimulation of chromosome transfer experiments. All the donor bacteria, including those carrying sex factors (such as F) which are derepressed with respect to their conjugal function, showed stimulation of chromosome transfer when newly transferred. The stimulation of transfer in the crosses involving the HFCT donor cultures was variable, probably due to the double sub-culturing of the HFCT donor preparation.

The greatest effect of new infection on the production of recombinants is shown by Col Ib drd. As with the effects of U.V. irradiation, there was frequently a 100-fold increase in the total number of recombinants yielded by this plasmid. The properties of the Col Ib drd plasmid therefore seemed to warrant a more detailed investigation.

3-B The properties of donor strains harbouring a newly transferred Col Ib drd plasmid

The fertility-derepressed mutant of Col Ib is readily transferred to recipient bacteria by conjugation, the kinetics of its transfer being almost identical to those observed in an HFCT donor culture of Col Ib. Transfer exceeds 90% within 15 minutes of mating, Figure 6. It is easy to prepare donor bacteria which have a newly transferred plasmid when transfer is so efficient, and, therefore, to examine

TABLE 17

A comparison of the effects of new infection on chromosome transfer by isogenic donor strains harbouring various plasmids

The total number of recombinants/ml.*

Sex factor	Standard Cross	Newly infected donors	Increase
F	5.7×10^3	1.7×10^5	X 29
F'lac	8.1×10^5	1.6×10^6	X 2
Col V2	5.8×10^2	6.4×10^3	X 11
Col V3	5.6×10^2	1.1×10^4	X 20
Col B3	5.1×10^3	2.2×10^5	X 41
Col VB	8.8×10^4	1.6×10^5	X 2
R1 drd 19	2.9×10^4	6.4×10^4	X 2
R192 drd F7	9.0×10^2	1.8×10^4	X 20
Col Ib drd	1.1	134	X 121
R64 drd 11	6.2×10^2	3.8×10^4	X 61
R144 drd 3	5.0×10^3	2.5×10^5	X 49

$$\left[\underline{\text{metB nalA}} \times \underline{\text{his trp spcA}}\right] \times \underline{\text{metB strA}}$$

* at least 1,000 recombinants scored per cross.

TABLE 18

A comparison of the effect of new infection on the fertility of Col B$^+$ donor strains

The total number of met$^+$B strA recombinants/ml.*

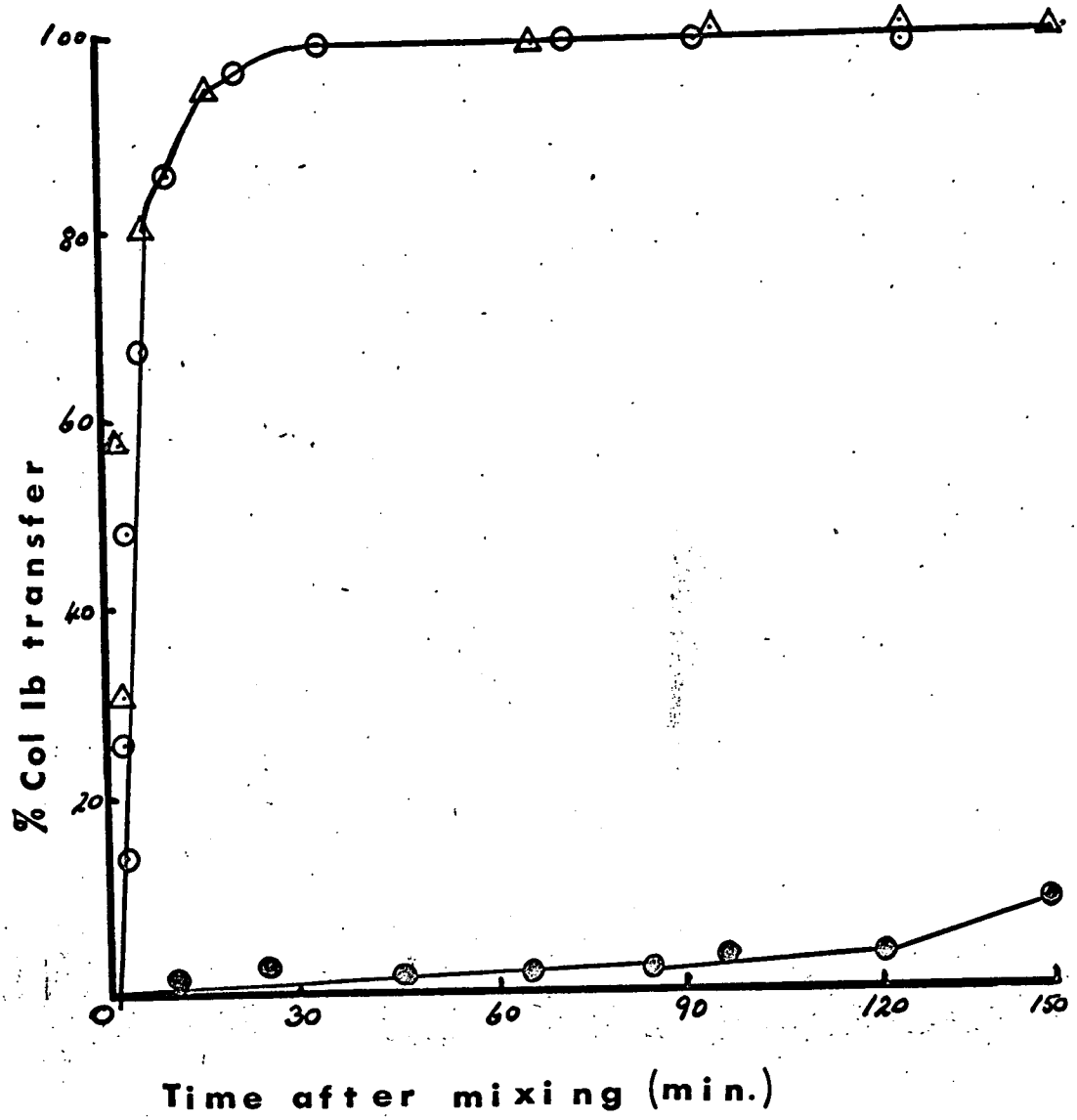
Sex factor	Standard HFCT cross	Newly infected donor	Increase
Col B1	3.4×10^2	2.8×10^3	X 8
Col B2	2.9×10^2	2.8×10^3	X 10
Col B4	6.6×10^2	9.2×10^3	X 14

$$\left[\left(\underline{\text{metB nalA}} \times \underline{\text{metB}}\right) \times \underline{\text{his trp spcA}}\right] \times \underline{\text{metB strA}}$$

* At least 1,000 recombinants per cross were scored.

Figure 6.

The transfer of Col Ib.



whether or not the process of new infection can derepress other plasmid functions. The derepression of the conjugal transfer system in an HFCT donor culture also leads to an increased synthesis of colicin, as measured by lacunae formation, Table 19. In addition, there is a loss of viability of the cells of an HFCT culture.

Bacteria harbouring the Col Ib drd plasmid also show an increased synthesis of colicin even when stably infected, the basal level of lacunae formation being higher than in the case of the wild type repressed plasmid. This level is increased still further upon new infection. The viable counts of cultures of Col Ib drd donors are frequently as much as 50% lower than the total counts (see Table 19). These pleiotropic effects are shown by several derepressed fertility mutants of Col Ib type plasmids (Dowman & Meynell, 1970). Measurements of the amount of plasmid DNA and of a plasmid-determined enzyme led these authors to conclude that the increased colicin synthesis does not result from an increased number of plasmid genomes per donor cell. However, the possibility exists that there may be a period of multiplication of the plasmid after it is newly transferred, as has been found in the case of an F'lac factor, (de Haan & Stouthamer, 1963).

The presence of a Col Ib plasmid in a bacterium greatly increases the cell's resistance to damage by ultraviolet light (Howarth, 1965; Takano, 1966; Walsh & Meynell, 1967). This increased resistance will also protect a recA strain from small doses of radiation, up to 100 ergs/mm^2. When this dose is exceeded a recA Col Ib strain dies at approximately the same rate as a recA F$^-$ strain.

TABLE 19

The derepression of colicin synthesis on new infection

The measurement of the numbers of lacunae/ml given by different preparations of Col Ib

Donor culture	Viable count of donor bacteria	Number of lacunae/ml	Transfer of Col Ib
Col Ib	2.1×10^8/ml	8.6×10^2	1.0%
Col Ib LFCT	2.0×10^8/ml	2.9×10^3	3.8%
Col IB HFCT	1.6×10^8/ml	4.6×10^5	99%
Col Ib drd	1.1×10^8/ml	7.8×10^4	98%
newly infected Col Ib drd	1.0×10^8/ml	1.0×10^6	100%

Lacunae production: Serial dilutions of a newly infected Col Ib drd donor culture were made together with the control Col Ib cultures, and 0.1 ml samples were plated in triplicate in a lawn of Col-sensitive strA indicator bacteria, on streptomycin nutrient agar plates. The plates were incubated at 37^o, and the lacunae scored after 6 hrs.

The U.V. survival of strains which harboured a newly transferred Col Ib drd plasmid was measured. The results are shown in Figure 7. Bacteria newly infected with the Col factor have an increased resistance compared to a stably infected strain. Furthermore, the protection against U.V. damage in a newly infected recA strain raises the survival to that of a newly infected rec$^+$ strain. Newly infected recA bacteria can survive U.V. doses of up to 1,000 ergs/mm^2 before they start to die at an appreciable rate, but at these large U.V. doses there is considerable damage to the plasmid which often shows deletions involving several characters on transfer to another cell (Moody, unpublished data). This could be the reason for the loss of the U.V. protection if the plasmid is so damaged as to be non-functional. It is interesting that cells newly infected with Col Ib drd plasmids are apparently less susceptible to the induction of colicin synthesis by ultraviolet light, perhaps because induction is already approaching the maximum. The protection that Col Ib affords an irradiated cell suggests that perhaps this plasmid can specify the synthesis of a repair enzyme, although this is probably not an excision enzyme since Col Ib does not confer protection on uvr$^-$ bacteria (Evenchik et al, 1969; Moody, unpublished data). The synthesis of this proposed enzyme could be induced by new infection, so giving rise to the increased resistance to U.V. damage.

Since newly infected recA strains are protected from U.V. death there might also be some restoration of their recombination-deficiency. The results obtained in Hfr crosses with a newly infected recA recipient strain are shown in Table 20. There is at least a 100-fold

Figure 7.

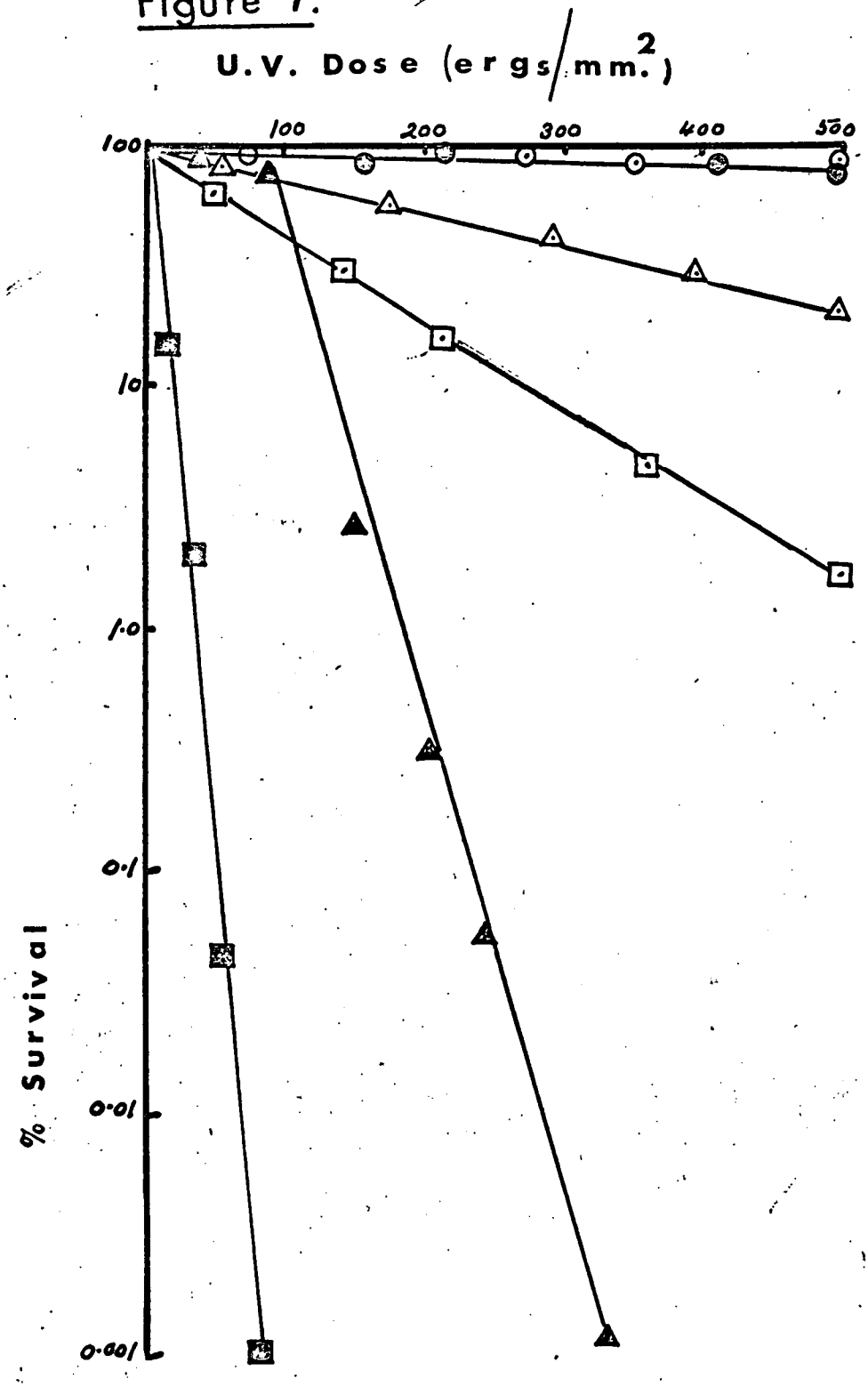

U.V. Dose (ergs/mm.²)
100
200
300
400
500
100
10
1.0
0.1
0.01
0.001
% Survival
rec⁺,
recA; Col Ibdrd new.
rec⁺,
recA; Col Ibdrd stable.
rec⁺,
recA; F⁻.

TABLE 20

Recombinant production by rec$^+$ and recA recipient bacteria newly infected with Col Ib drd

$\not$The frequency of met$^+$B nalA recombinants per 100 input Hfr

Sex factor in recipient	rec$^+$ recipient	recA recipient
None	8.2×10^{-1}	1.1×10^{-5}
Col Ib	7.9×10^{-1}	1.2×10^{-5}
Col Ib drd	8.3×10^{-1}	1.0×10^{-5}
newly infected Col Ib drd	8.0×10^{-1}	4.1×10^{-3}
*newly infected Col Ib uvp-2	8.6×10^{-1}	2.0×10^{-5}

Hfr P13 cys his X W1655 metB nalA F$^-$

* Col Ib mutant which fails to confer resistance to ultraviolet light (see Figure 8)

$\not$ At least 500 recombinants scored per cross.

increase in the number of recombinants produced by a recA recipient which has been newly infected with Col Ib drd as compared to the stably infected recA strain. Thus there is a partial restoration of the ability to produce recombinants in these newly infected recombination-deficient strains, although it does not approach the wild type level.

3-C Discussion

Bacteria newly infected with transmissible plasmids behave in a manner similar to stably infected bacteria which have been irradiated with ultraviolet light. The conjugal transfer systems of repressed plasmids can be derepressed by either low dozes of U.V. irradiation (Monk & Clowes, 1964b) or by new infection in an HFCT donor culture (Stocker, Smith & Ozeki, 1963). This results in the efficient transfer of the temporarily derepressed plasmid to recipient bacteria. The same conditions also lead to an increase in the number of cells that commence lethal colicin synthesis in a colicinogenic strain.

Monk & Clowes (1964b) envisaged the regulation of plasmid functions through cytoplasmic repressors, whereby the inactivation of repressor function by U.V. irradiation, or its absence in newly infected strains, leads to the expression of plasmid genes in a manner similar to the U.V. induction (Jacob & Monod, 1961) or the zygotic induction of a λ lysogen (Jacob & Wollman, 1956a; Wollman & Jacob, 1957).

One might expect that the various plasmid functions would be regulated independently but this does not seem always to be the case.

A fertility derepressed mutant of Col Ib-type plasmid, R144 drd, also showed an increased synthesis of colicin, as judged by the size of the inhibition zone (Meynell et al, 1968). This increased colicin synthesis is apparently not due to an increased number of plasmids per cell (Dowman & Meynell, 1970). An intimate relationship between the transfer genes of a colicinogenic factor and the genes concerned with colicin synthesis is also found in the sex factor Col V2. This is a naturally derepressed F-like colicinogenic factor. The conjugal transfer system of Col V2 is subject to the repressor produced by a repressed Rfi$^+$ factor, R222 so that, in a strain harbouring both Col V2 and R222, virtually no Col factor transfer takes place. At the same time the amount of colicin produced is only 25% that of the same strain carrying the Col V2 alone (Moody, unpublished data). This could mean that at one time there might have been a functional relationship between the transfer genes and the genes concerned with colicin synthesis; for example, colicin V2 might have evolved from mutant pilus sub-units. In contrast, if an F'lac factor is substituted for the Col factor, the fertility system is repressed as before but the synthesis of β-galactosidase is completely unaffected by the R-factor repression (Moody, unpublished data).

It is proposed that the transfer of chromosomal material is another inducible plasmid function. The stimulation of chromosome transfer by ultraviolet irradiation (Hayes, 1952a, 1953b; Rajchert-Trzpil & Dobrzański, 1968; Evenchik et al, 1969) (see also Tables 14 & 15) and by new infection (see Tables 17 & 18) can be

accounted for by the derepression of this plasmid function. The increased interaction of transmissible plasmids with the bacterial chromosome after U.V. irradiation has been attributed to the excision of a damaged strand of DNA in a region of sex factor homology, thus exposing a single stranded region which is more readily recognised by the sex factor (Evenchik et al, 1969). This model has been criticised in Experimental Section 2-B on the basis of the results obtained with Col Ib drd, a plasmid which seems to lack homology with the chromosome and yet is stimulated by ultraviolet light. These criticisms are strengthened by the data obtained in Experimental Section 3-A. Donor bacteria which have a newly transferred plasmid show a stimulation of chromosome transfer similar to that of the U.V. induced donors. Here there is no reason to suspect that there is any damaged DNA to be excised in the regions of sex factor homology. A reasonable alternative model, therefore, is the induction of some plasmid-specific function which can be derepressed by either U.V. stimulation or by new infection. The derepression leads to an increased interaction between the plasmid and the chromosome which results in the transfer of chromosomal genes during conjugation.

However, in a standard cross involving an autonomous plasmid, this postulated plasmid function must be assumed to interact with the bacterial REC system, since both systems are required to maintain the normal frequency of recombinants. If the bacterial REC system is defective, as in recA donors, the frequency of recombinants drops to the levels shown in Table 13. This low level of recombinants

is characteristic of the frequency of recombinants produced by Col Ib$^+$drd donors, regardless of their recombination proficiency. The results obtained in Experimental Section 1-C suggest that the transfer of chromosome by Col Ib drd plasmids is by a mechanism which is totally independent of the bacterial REC system, and so may be entirely mediated by a plasmid-specified function. If this is true, the increase in chromosomal transfer shown by Col Ib drd intermediate donors should also work in recA donor strains.

The results obtained with some newly infected recA (Table 21) donors are shown in Table 22. The low numbers of recombinants sired in the majority of crosses involving recA donors made it necessary to repeat the crosses many times to check the reproducibility of the results. Normally, donor cultures were set up in triplicate and all crossed with the same recipient. The result of each experiment was taken as the average of the three donor cultures, and repeated at least five times.

Surprisingly, new infection by F or F' plasmids turned out to provoke as high a relative increase in the number of recombinants produced by the rec$^-$ donors as had been found in the case of rec$^+$ donors (Table 17) although, of course the absolute number of recombinants was enormously greater in the rec$^+$ crosses. In the latter crosses, the stimulation of chromosome transfer does not appear to be directly attributable to induction of the proposed plasmid-specific function, since the REC system is clearly necessary for efficient transfer. To investigate the contribution of the plasmid-specific function to normal transfer would require analysis

TABLE 21

The strains used to demonstrate the stimulation of chromosome transfer in newly infected recombination deficient donor bacteria

The primary donor strains were W1655 *nalA recA* F^- derivatives infected with the plasmids listed in Table 5. The origin of this recombination-deficient strain is described in Table 6.

The secondary donor strain was a recombination-deficient mutant derived from JC5455 by Dr. N. S. Willetts. It is designated JC5466 *his trp spcA recA* F^-

The new infection of secondary donors was achieved as follows:

Primary donor		Secondary donor		Recipient
	X		X	
metB nalA recA		*his trp spcA recA* F^-		*metB strA* F^-

TABLE 22

The effect of new plasmid infection on the number of recombinants for chromosomal genes sired by recombination-deficient (recA) isogenic donor strains

The total number of recombinants per ml *

Sex factor	Standard cross (stably infected donors)	Newly infected donors	Increase
F	2.0	60.0	X 30
F'lac	2.4	9.6	X 4
R1 drd 19	1.6×10^4	3.2×10^4	X 2
Col Ib drd	0.67	69.0	X 103

[metB recA nalA X his trp recA spcA] X metB strA

* At least 200 recombinants were counted in each cross

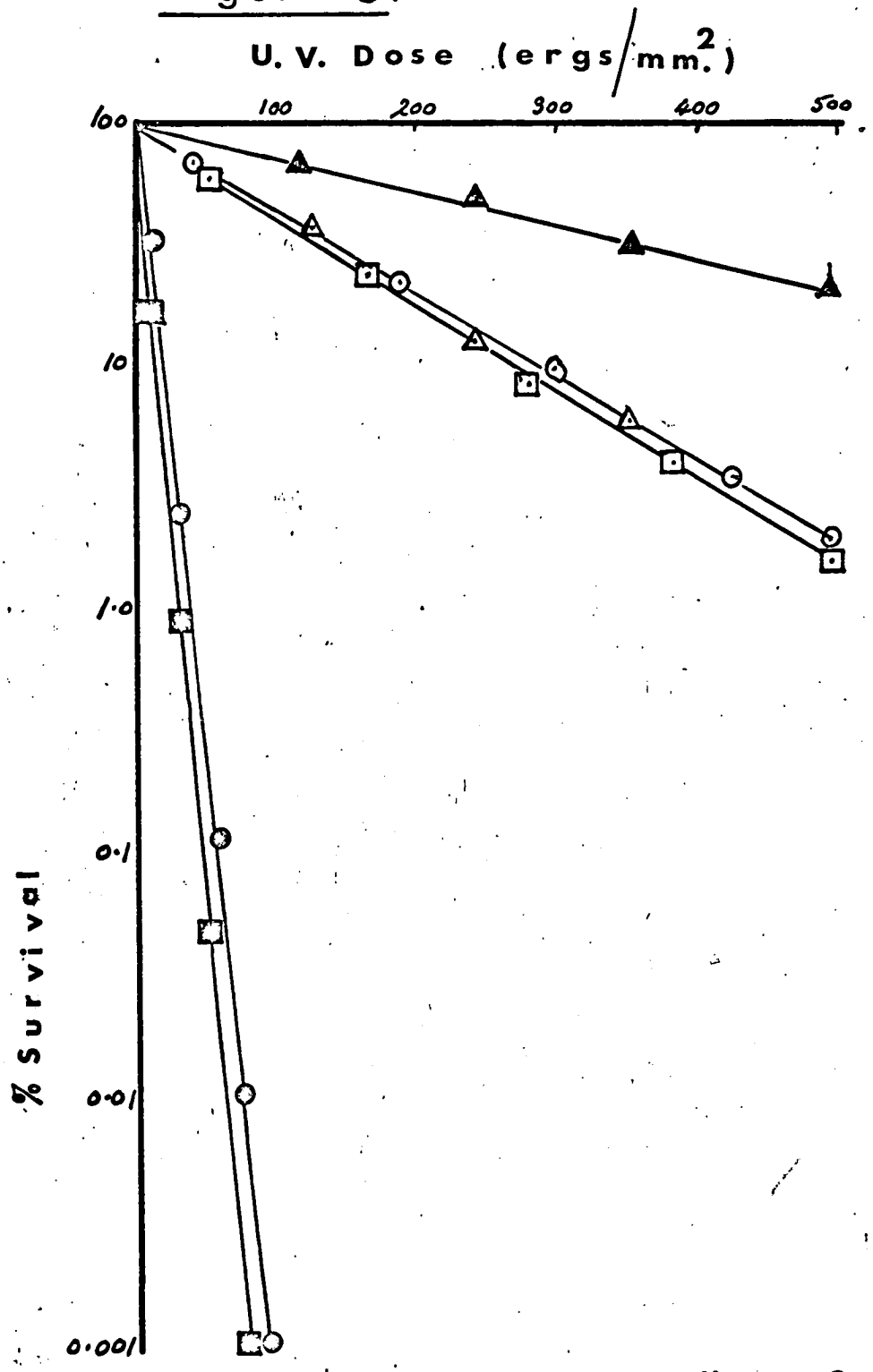
of the behaviour, in *rec*$^+$ strains, of mutants of the *F* factor which are unable to stimulate chromosome transfer on new infection of *rec*$^-$ bacteria. Such mutants have not yet been isolated.

The induction of a plasmid specific function revealed in Table 22, although striking, occurs at such a low level that it is only just detectable in *recA* donors. However, the levels of chromosome transfer by both *Col Ib drd* and by *R1 drd 19* donor strains are directly related to the induction of this plasmid function. Chromosome transfer by these two plasmids is independent of the REC system.

The properties of bacteria harbouring a newly transferred *Col Ib drd* factor made it very tempting to explain the U.V. protection effect in terms of a plasmid-specific recombination enzyme. Enzymes involved in recombination and repair mechanisms have many similar functions to perform, and it seemed probable that, in *Col Ib*, these functions might have an enzyme in common. Unfortunately, this is not the case.

There are mutants of the *Col Ib* plasmid which have lost their ability to protect a bacterial cell from U.V. damage (Howarth-Thompson, 1969). The experiments performed with *Col Ib drd* were repeated using one of these mutants (*Col Ib uvp-2*). This *Col* factor confers no resistance to ultraviolet irradiation, even when newly transferred (see Figure 8). Newly infected *recA* strains are neither protected from U.V. death, nor does the factor partially restore their recombination-proficiency as does the wild type *Col* factor (see Table 14). However, donor strains harbouring a newly

Figure 8.


U. V. Dose (ergs/mm.²)
100
200
300
400
500
100
10
1.0
0.1
0.01
0.001
% Survival
rec⁺,
recA; Col Ib uvp-2 new.
rec⁺,
Col Ib uvp-2 stable.
rec⁺,
recA; F⁻.
rec⁺ Col Ib drd new.

transferred Col Ib uvp-2 factor show the same degree of stimulation of chromosome transfer as the derepressed mutant, Col Ib drd. It is therefore reasonable to assume that the Col Ib genes which are responsible for the U.V. protection are not the plasmid's recombination enzymes, or at least not those responsible for its interactions with the chromosome.

CHAPTER 5

THE REGULATION OF PLASMIDS

1-1 Many plasmids pass through two stages on entry into a new bacterial host. Immediately upon infection of a new cell there is expression of some plasmid functions which are ultimately switched off. When plasmids enter the second stage they either multiply independently of the chromosome but in parallel with their host cell or they may integrate into the chromosome. If a plasmid integrates it is then replicated as part of the bacterial genome.

An autonomous plasmid establishes a stable relationship with the bacterial cell. It keeps pace with cell division with probably less than two plasmid copies per bacterial chromosome. This conclusion was drawn from the fact that when F^+ or F'lac donors are superinfected with an homologous plasmid like an F'gal, very quickly two cell lines are established, each carrying only one of the two plasmids (Scaife & Gross, 1962). A rapid segregation would not be expected if there was a large pool of plasmids per cell. The number of copies of an F'lac factor per cell was also estimated at one or two from the levels of β-galactosidase produced (Jacob & Monod, 1961). There may be a period of multiplication of an F' factor immediately after its introduction into a new host cell (de Haan & Stouthamer, 1963). Any model proposed to explain the control of replication of transmissible plasmids must also account for the very low frequency of loss of the plasmid, indicating not only that replication of the plasmid is controlled but also that the segregation of daughter replicas is

precisely co-ordinated with cell division. The model of Jacob & Brenner (1963) incorporates both these aspects of plasmid propagation.

1-2 The replicon hypothesis

According to the model of Jacob & Brenner (1963), the sex factor F is a replicon, which they define as an independent unit of replication. The replicon hypothesis of Jacob, Brenner & Cuzin (1963) states that all replicons, including the bacterial chromosome and many sex factors are controlled by their particular gene product, the initiator. This initiator interacts specifically with a particular site of the replicon, the replicator, the result of this interaction being the initiation of a new round of DNA replication at the replicator. For purposes of segregation and transfer the model also assumes that replicons attach to sites on the cell membrane which increase in number or in area as the cell grows (Marvin, 1968). While accepting that the attachment-site hypothesis accounts for many features of the behaviour of plasmids, Pritchard, Barth & Collins, (1969) point out that some are not so readily interpreted in these terms. They propose a cytoplasmic system of negative control whereby reinitiation is prevented by a repressor determined by a gene located very close to the replicator or 'origin'. Increase in the volume of the growing cell would reduce the concentration of this repressor below a critical threshold so that a new round of replication is initiated and the concentration of repressor is doubled by gene duplication (Pritchard, 1968, 1969; Pritchard et al, 1969).

Some of the functions involved in the replication of established sex factors were defined by Jacob et al (1963) by the isolation of conditional lethal mutants of an F'lac$^+$ factor which are unable to replicate at 42°. When lac$^-$ cells carrying these mutants are spread on an indicator medium, they produce lactose fermenting colonies at 30° but not at 42°, due to loss of the F'lac$^+$ factor at the higher temperature. In fact, two genetic classes of sex factor replication mutants were obtained, in one of which the sex factors themselves are mutant (for example, F'ts$_{62}$lac$^+$ and F'ts$_{114}$lac$^+$) and are unable to replicate at 42° regardless of their F$^-$ host background. The second class of mutant involves the bacterial chromosome (for example, ts$_{112}$) so that it is unable to maintain a wild type F' factor when incubated at 42°. The isolation of these two classes of mutations suggests that both plasmid and bacterial components are involved in the replication of plasmids. However, the F'ts factors are able to multiply at the high temperature if they are harboured in the same cell as a wild-type sex-factor, suggesting that the defective plasmid component is a cytoplasmic product (Jacob et al, 1963).

If donor strains carrying either class of mutation are incubated at 42° it is possible to rescue the F' factor by integrating it into the chromosome. If the donor chromosome is deleted for the bacterial region carried on the F' factor, this can be used to isolate a wide variety of Hfr strains, by selecting colonies which retain the sex factor at 42°. Using this highly selective technique Hfr donors have been isolated from F'ts$_{114}$lac$^+$ strains (Broda, pers. commun.) and from ts$_{112}$ mutants harbouring a wild-type sex-factor (Moody, unpublished data).

The Hfr donors isolated by either technique are highly fertile when used in crosses with F^- recipients at both 30° and 42°. The transfer of the cytoplasmic $F'ts_{114}lac^+$ factor is also normal at both temperatures. Whatever the defect is in $F'ts_{114}lac^+$, its defective replication apparently has no ill effects on its own transfer or its transfer of chromosome when integrated. The Jacob et al (1963) model proposes that a replicon should occupy a specific site on the cell membrane. In the case of the sex factor, its membrane attachment site would also be the site at which conjugal union occurs so that, whether transfer to a recipient bacterium occurs through a sex pilus (Brinton, 1965, 1967; Brinton et al, 1964) or by the mechanism proposed by Curtiss (1969) the sex factor would be in a favoured position. According to this hypothesis the simplest interpretation of the effect of the ts_{112} bacterial mutation is that the membrane is somehow altered at 42°, so preventing the stable attachment and replication of the sex factor. It is therefore surprising that the rescued F' factors in a ts_{112} mutant are Hfr at 42°, since, being unattached, it should be unable to respond to sexual contact with an F^- recipient which is supposed to trigger transfer at the attachment point.

1-3 The phenomenon of integrative suppression

When the F sex factor is integrated into the bacterial chromosome its autonomous replication system is non-functional and it is replicated as part of the bacterial genome by the chromosomal replication mechanism (Caro & Berg, 1969; Cuzin & Jacob, 1967; Hirota, 1960). This is partly based on the observation that the

integrated sex factor in an Hfr strain is not susceptible to curing by acridine dyes (Hirota, 1960). However, many plasmids are not susceptible to acridine curing (Clowes, Moody & Pritchard, 1965; Ozeki, 1960) and there is no evidence to suggest that these plasmids can integrate at any appreciable frequency. When an F factor regains its autonomy from an Hfr strain to form an F^+ or an F' factor, its autonomous replication system becomes functional once more. This F-replication system can replicate larger genetic element than the sex factor alone. Many F' factors carry considerable amounts of bacterial chromosomal material, and could be considered as additional chromosomes in the cell. This reasoning was carried to its extreme by Nishimura, Caro, Berg & Hirota (1971) who designed experiments to see if the F-replication system could replicate the entire bacterial genome under conditions where the bacterial initiator-replicator system was non-functional. They presented evidence for a new type of suppression of a temperature-sensitive mutation affecting the initiation of DNA replication which requires the integration of a functional episome into the chromosome of the ts_{DNA} mutant; there was no complementation of the initiation defect if the sex factor was autonomous. When temperature-resistant revertants were picked from a $ts_{DNA}F^+$ donor strain nearly all were found to have the F sex factor integrated at a number of sites on the chromosome and to have retained the original ts_{DNA} mutation. Since these revertants proved to be sensitive to acridine orange and ethidium bromide at 42°, but not at 30°, it is claimed that at 42° the initiation of chromosome replication is an F-specified

function. Mutations involving replicative functions other than initiation are not suppressed by sex factor integration.

All revertants resulting from integrative suppression are, of course, Hfr donors. However, F integration per se is not sufficient to effect the reversion, since not all Hfr strains isolated at 30° are able to grow at 42°. The phenomenon does not take place in recA strains. Clearly, as the authors point out, the selection of integratively suppressed revertants from a ts_{DNA} F^+ donor is a useful tool for isolating Hfr donors.

The suppression of a ts_{DNA} initiator mutant is also possible with a sex factor which is defective in its own replication, for example $F'ts_{114}lac$ and $F'ts_{62}lac$, although there is no complementation of either defect when the sex factor is autonomous. However, although either replication-defective factor can, on integration, permit chromosome replication at 42°, the resulting strains are not Hfr donors and appear to be completely sterile (Moody, unpublished data). It is unlikely that this infertility reflects an essential joint-role of the two replication systems which is required for chromosome transfer, as the defective factors are capable of autonomous transfer. Since integration alone is not sufficient to give integrative suppression, it is claimed that some internal rearrangements within the integrated sex factors may be necessary (Nishimura et al, 1971). These proposed rearrangements could perhaps cause some defects in the plasmid's transfer genes. The revertants are sensitive to the male-specific phage M.S.2 indicating that if they are transfer deficient mutants they would have to be

tra G or tra I mutants. The tra I gene is almost certainly responsible for the transfer of the sex factor itself (Willetts, pers. commun.). Tra I mutants of an F'lac factor will promote the transfer of a non-transmissible plasmid like Col E1 to a recipient, but the mutant F'lac is not transferred (Alfaro & Willetts, pers. commun.). It is possible that, in the case of these mutant sex factors, integration disrupts one of these genes.

Appendix 3

See the attached publication

The elimination of extrachromosomal elements in thymineless strains of Escherichia coli K12

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Genet. Res., Camb., (1965), 6, pp.147-152.

In this publication no explanation was offered to explain the observed curing of plasmids by thymine deprivation. Thymine-requiring mutants of *E. coli* are apparently much more sensitive than wild-type to the presence of cobalt and nickel salts (Devoret, pers. commun.). Cobalt and nickel salts were first used to eliminate the *F* sex factor. It is possible that plasmids in *thyA* strains could be more susceptible to curing by the trace amounts of these elements in the minimal medium used in these experiments.

Alternatively, when thymine-requiring bacteria are starved of thymine, interruptions appear in the covalently closed circular DNA of the sex factor (Freifelder, 1969). The circles of sex factor DNA are lost at a rate which is proportional to the molecular weight of the sex factor, suggesting that the interruptions do not occur at a unique site on the sex factor DNA molecule. These breaks are probably single-stranded interruptions, which Freifelder (1969) hypothesizes would normally be repaired. Thymine starvation perhaps inhibits a repair enzyme, or possibly a DNA ligase (Olivera & Lehman, 1967) which would normally repair these naturally occurring breaks, so that the sex factor is eliminated.

Genet. Res., Camb. (1965), **6**, *pp.* 147–152
Printed in Great Britain

SHORT NOTES

The elimination of extrachromosomal elements in thymineless strains of *Escherichia coli* K12

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(*Received* 27 *November* 1964)

INTRODUCTION

The concept that the *F* sex factor of *Escherichia coli* K12 exists in an extrachromosomal state in F+ cells, and in a chromosomally-integrated state in Hfr cells is supported by the demonstration that it may be eliminated from F+, but not from Hfr males. This elimination of *F*, first reported in the presence of cobalt and nickel salts, was found to be accomplished more effectively by treatment with acridine dyes. Under optimal conditions virtually 100% of F+ cells were 'cured' of *F* to become F− (see Hirota 1960), whereas no curing was found in Hfr strains.

Since that time extrachromosomal elements (or plasmids) other than *F* have been extensively investigated, particularly in *E. coli* and related strains of Enterobacteriaceae. These elements include the *colicin factors* (or *col* factors) that control the maintenance of protein-like antibiotics or *colicins* in colicinogenic (*col*+) strains. It has been suggested that although some of these *col* factors are infective and may be transferred in the absence of chromosomal characters, they may have a chromosomal location in the majority of the cells of a culture of a stable colicinogenic strain: (see Smith, Ozeki & Stocker, 1963).

A specific chromosomal site has in fact been claimed for *colE*1, but this conclusion does not appear to be supported by more extensive genetic experiments (see Clowes, 1964). Other genetic crosses suggest that other *col* factors are transferred as extrachromosomal elements and a concept of colicin factors as self-regulating extrachromosomal genetic elements has been proposed (see Monk & Clowes, 1964*a*, *b*) to account for their genetic features. However, in spite of their apparent non-chromosomal nature, the elimination or 'curing' of any *col* factor does not appear to have been accomplished (Ozeki, 1960).

This paper reports a method whereby elimination of *col* factors and of other extrachromosomal elements may be achieved from thymineless mutants under conditions of thymine limited growth.

MATERIALS AND METHODS

Bacterial Strains. The derivation and characteristics of the strains used in this study are listed in Table 1. The methods of isolation of colicin resistant and colicinogenic derivates have been described elsewhere (Monk & Clowes, 1964*a*).

Media. In addition to basic media described by Monk & Clowes (1964*a*), specific media

included: *Low phosphate medium*, the 'TG' medium of Echols, Garen, Garen & Torriani (1961) and *M9 medium*, made up as Na_2HPO_4 (anhydr.) 6 g., KH_2PO_4 (anhydr.) 3 g., NaCl 0·5 g., NH_4Cl 1·0 g., $MgSO_4$ 0·001 M, $CaCl_2$ 0·0001 M, Glucose 4 g., water to 1 l.

Techniques. Colicinogeny was tested by the triple overlay technique (see Monk & Clowes, 1964*a*). *ColE1*$^+$ colonies were tested in the presence of the colicin I factor by using a strain resistant to colicin I (510) as an indicator. Similarly, when testing for *colI*$^+$ colonies in the presence of the *colE1* factor, an E1-resistant indicator (511) was used.

F status of strains was checked by testing for resistance (F^-) or sensitivity (F^+ or Hfr) to the male-specific phage μ by a modified screening method (Monk & Clowes, 1964*a*).

Acridine orange treatment followed the method of Hirota (1960). A log phase culture at *c.* 10^8/ml. was inoculated to produce a cell density of about 10^4/ml. in broth at pH 7·6 containing acridine orange at concentrations from 40 to 100 μg./ml. The culture was incubated with aeration overnight, during which the cell density increased to about 10^8/ml. Dilutions were then plated for single colonies which were individually scored for colicinogeny by the triple overlay technique.

Isolation of thymineless mutants was achieved by a simplified version of the Okada, Yanagisawa & Ryan (1961) technique. A log phase culture (*c.* 2×10^8/ml.) was diluted to about 500 cells/ml. into M9 medium (supplemented with appropriate growth factors) containing thymine and aminopterin (both at 200 μg./ml.), and incubated for 48 hours at 37°. Samples were then plated on nutrient agar supplemented with thymine (200 μg./ml.) and clones of thymineless mutants were recognized by their inability to be transferred by replica plating to nutrient agar. In most instances, 50 to 100% of the surviving clones are found to be thymine requiring, but in rather high concentrations, 40 μg./ml. being required for optimal growth.

Growth on limiting thymine. 0·1 ml. samples of an overnight culture (10^9/ml.) of a thymineless mutant in M9 medium supplemented with appropriate growth factors and containing 40 μg./ml. thymine, were subcultured into a series of 5 ml. volumes of M9 medium, supplemented with the same growth factors and various sub-optimal concentrations of thymine (0, 2, 10, and 25 μg./ml.), and incubated for 24 hours with aeration. Appropriately diluted samples were then plated for single colonies in soft agar containing 50 μg./ml. thymine on nutrient agar plates similarly supplemented with thymine. Colonies which appeared after overnight incubation were tested for colicinogeny by the triple overlay method, and a random sample of colonies picked from the overlay were tested for maleness (*F*) with μ phage, and in some instances for colicinogeny by a stab technique (see Monk & Clowes, 1964*a*).

RESULTS

(A) *Acridine orange treatment*

The first experiments involved a strain (557) carrying the plasmids *F*, *colI* and *colE1* which after standard acridine orange treatment at 50 μg. and 75 μg. was plated for single colonies and tested for each of the three factors. Among 80 colonies tested at either AO concentration, all were found to have lost the F sex factor, but to have retained both *col* factors (Table 2, lines 1 and 2).

A further series of experiments involved strains which were first made resistant to colicin, before acquiring colicinogeny. In this way it was hoped that 'cured' cells would remain insensitive to the action of the free colicin liberated in the medium by the non-cured majority. With a strain both colicinogenic for, and resistant to colicin E1 (521), no colonies cured of *colE1* were found among 1604 tested, although every one of ten colonies tested for *F* were found to have lost this factor (Table 2, line 3). A further strain, both colicinogenic and resistant to colicins I and E1 (551) was treated with AO

Table 1. *Derivation and characters of bacterial strains*

(a) *Strains derived from* Escherichia coli *K*12, 58–161, met⁻

Strain No.	*Characters*	*Origin*	*Strain No.*	*Characters*	*Origin*
501	F⁺ (See Monk & Clowes, 1964*a*)		516	F⁺*colE1-r*	501
			521	F⁺(*colE1*)⁺*colE1-r*	516
502	F⁻*str-r*		546	F⁻(*colI*)⁺*colI-r*(*colE1*)⁺*colE1-r str-r*	512
518	F⁻		551	F⁺(*colI*)⁺*colI-r*(*colE1*)⁺*colE1-r str-r*	546/501
510*	F⁻*colI-r str-r*	502	528	F⁻(*colI*)⁺(*colE1*)⁺	518
511†	F⁻*colE1-r str-r*	502	557	F⁺(*colI*)⁺(*colE1*)⁺	528/501
512	F⁻*colI-r colE1-r str-r*	510	566	F⁺(*colI*)⁺(*colE1*)⁺*thy*⁻	557

(b) *Strains derived from other* E. coli *K12 strains*

Strain No.	*Characters*	*Origin*
779	A′Hfr(*colE1*)⁺*thy*⁻	732 (Monk & Clowes, 1964*a*)
770	W1655 *met*⁻*lac*⁺ (*Flac*⁺)	(Scaife & Gross, 1962)
790	*met*⁻*pro*⁻*lac*⁻*thy*⁻(*Flac*⁺)	A K12 recombinant made *thy*⁻ and infected with *Flac* from 770
533	W3747 *met*⁻*lac*⁻ (*Flac*⁺*ade*⁺)	The original strain carrying F13 = *Flac*⁺*ade*⁺ (Hirota & Sneath, 1961)
564	W3747 (*F13*) *thy*⁻	533

* Original source of *colI* and colicin I is *Shigella sonnei* (P9) (Monk & Clowes, 1964*a*).
† Original source of *colE1* and colicin E1 is *E. coli K30* (Clowes, 1964).

Table 2. *Elimination of plasmids with acridine orange*

Strain No.	AO concentration (μg./ml.)	*F* Examined	*F* % 'curing'	*colI* Examined	*colI* % 'curing'	*colE1* Examined	*colE1* % 'curing'
1. 557	50	40	100	2756	0	—	—
2. 557	75	40	100	3598	0	4027	0
3. 521	50	10	100	—	—	1604	0
4. 551	40	80	100	2580	0	2782	0
5. 551	50	80	100	1985	0	2155	0
6. 557	100*	40	100	1683	0	1307	0
7. 770	40	40	100	—	—	—	—
8. 533	40	40	0	—	—	—	—

* Grown in low phosphate medium (McFall, Pardee & Stent, 1958).

concentrations of 40 and 50 μg. Again although all tested colonies had apparently been cured of *F*, none of many thousands tested had lost either *col* factor (Table 2, lines 4 and 5).

It is known that under normal conditions of growth, K12 strains are multinucleate. Moreover, recent experiments (Ozeki, unpublished) suggest that replication of colicin factors occurs within a few minutes after transfer, thereby rendering heavily labelled (^{32}P) colicin factors stable to inactivation by ^{32}P 'suicide' unless the recipient cells are very rapidly frozen after infection by the colicin factor. In an attempt to limit the number of *col* factors and the number of nuclei per cell, the strains were grown on a low

phosphate medium, which has been shown to result in uninucleate cells (McFall, Pardee & Stent, 1958) prior to treatment with acridine orange at 100 μg./ml. Even under these conditions (Table 2, line 6) which produce 100% *F* 'curing', no (< 0·075%) curing of either *col* factor occurred.

(B) *Thymine limitation*

Thymineless derivatives of various plasmid-infected K12 strains were grown for 24 hours under conditions of sub-optimal thymine supplementation, and the surviving colonies were tested for retention of plasmids, with the results shown in Table 3. Strain 566, a thymineless derivative of strain 557 (carrying *F*, *colI* and *colE1*) was grown in

Table 3. *Elimination of plasmids under thymine deprivation*

Strain No.	Concentration of thymine (μg./ml.)	*F*		*colI*		*colE1*	
		Examined	% 'curing'	Examined	% 'curing'	Examined	% 'curing'
1. 566	10	70	100	1311	0	2533	50·9
2. 566	2	40	100	1331	4·4	1350	99·2
3. 566	2	40	100	1232	4·0	831	99·9
4. 779	10	40	0	—	—	1529	79
5. 564	10	28	100	—	—	—	—
6. 564	10	40	100	—	—	—	—
7. 566	25	80	50	—	—	—	—
8. 564	25	80	52·5	—	—	—	—
9. 790	25	80	49	—	—	—	—

medium containing 10 μg./ml. thymine. There was 100% loss of *F*, and about 50% loss of *colE1*, but no loss of *colI*.

The effect of more severe deprivation of thymine (2 μg./ml.), and of its complete absence from the medium were tested. From an inoculum of 10^7 thymineless cells/ml., no survivors (< 10 cells/ml.) were isolated after 24 hours' incubation in the absence of thymine. However at 2 μg./ml. thymine, the final cell concentration after 24 hours was *c.* 10^8 cells/ml. Among these cells, loss of all three plasmids was observed (Table 3, lines 2 and 3). All the examined clones were F^-, over 99% had lost *colE1*, and 4·0% were now 'cured' of *colI*.

The elimination of each colicin factor appeared to occur independently. Thus at 2 μg./ml. of thymine, less than 1% of the survivors retained *colE1*, nevertheless among those cured of *colI*, one out of fifty cells retained *colE1*. All the clones which had lost all three factors *F*, *colI* and *colE1* were found to remain *thy*$^-$, *met*$^-$ and *lac*$^+$.

The effects of thymine deprivation on an integrated *F* factor and a colicin factor were examined by subjecting an Hfr *colE1*$^+$ strain to this treatment. In such a strain (779), the expected elimination of *colE1* occurred but no elimination of *F* was found in 40 colonies examined.

(C) *Elimination of other plasmids by growth in limiting thymine*

The *F* factor of K12 has been shown to be capable of incorporating segments of chromosomal material, giving rise to '*F prime*' heterogenotic strains (see Driskell-Zamenhof, 1964). Some of these F prime strains, for example 770, a strain carrying an F prime factor incorporating a *lac*$^+$ marker (*Flac*$^+$) is completely cured of both *F* and

lac+ after standard acridine orange treatment (Table 2, line 7). In contrast, another strain (533) with an F prime (*F13*) carrying a number of chromosomal genes (Hirota & Sneath, 1961) was resistant to curing by this method (Table 2, line 8). However, when a thymineless derivative of 533 (564) was grown in limiting (10 μg./ml.) thymine, all the isolated clones were found to have lost this plasmid as judged from inability to plate μ (Table 3, lines 5 and 6). Four of these presumed 'cured' strains were examined for their ability to transfer *lac*+. All were now found to be unable to transfer this factor (<1%) to an F$^-$*lac*$^-$ strain after 2 hours contact compared to a 100% transfer achieved by the parental 564.

When the three strains carrying *F*, *Flac* and *F13* respectively were grown under less severe thymine deprivation (25 μg./ml.) which did not cure every cell of *F* factors, these strains all showed the same sensitivity to thymine 'curing', the factor being lost from about 50% of the cells in each case (Table 3, lines 7, 8 and 9).

DISCUSSION

In confirmation of the results of Ozeki (1960), it was found that colicin factors, in this instance *colI* and *colE1*, were refractory to elimination by acridine orange treatment. No elimination of either *col* factor was found from strains under conditions which led to a complete elimination of the *F* sex factor.

In contrast, when thymineless strains were grown in limiting concentrations of thymine, both colicin factors were eliminated. The colicin E1 factor was lost in up to 99·9% of the population, whereas *colI* was lost in a smaller proportion, less than 5% of the surviving clones.

Since thymine deprivation is known to induce lethal phage synthesis in λ-lysogenic strains and lethal colicin synthesis in colicinogenic strains (Sicard & Devoret, 1962; Luzatti & Chevalier, 1964), it might be suggested that 'curing' of the colicin factors occurs as a result of the induction of a genetic element that was initially chromosomally integrated. The kinetics of exponential death of colicinogenic and non-colicinogenic strains under conditions producing thymineless death and colicin induction have been studied. Luzzati & Chevalier (1964), using resting cells, found no difference in these kinetics, whereas Sicard (1964) found that there is a more rapid loss of viability in cells of a colicinogenic strain under these conditions. Our experiments were also performed with resting cultures so it is possible that colicin induction is occurring only in these cells undergoing thymineless death, and the surviving clones have not arisen from cells that have undergone a non-lethal induction. In any event, no elimination of λ was found from strains in which successful *col* factor elimination had occurred, nor was there any elimination of chromosomally-integrated *F* under these conditions of *col* factor elimination.

It may be concluded, therefore, that factors integrated in the chromosome are not eliminated in cells surviving thymine deprivation, so that the elimination of *col* factors under these conditions may be adduced as evidence of their non-chromosomal nature. This conclusion is in agreement with observations on the genetic transfer of *colI* and *colE1*, and supports the suggestions that all colicin factors are extrachromosomal under all conditions so far investigated (see Clowes, 1964).

There is, however, a striking difference in the susceptibility to elimination shown by various extrachromosomal elements. Growth in the presence of acridine orange eliminates wild-type *F* and certain F prime factors with great efficiency, but is without effect on colicin factors, and on a particular F prime, (*F13* of Hirota & Sneath, 1961). In 10 μg./ml. thymine, there is complete elimination of *F13*, *c.* 50% elimination of *colE1*, and no elimination of *colI*. When thymine deprivation is more severe (2μg./ml.), *colE1* elimination is increased to 99% and a small proportion of cells now lose *colI*, but the

two *col* factors appear to be eliminated independently, since about 2% of the cells which lose *colI*, retain *colE1*.

Curiously, although *F13* is apparently refractory to curing by acridine orange in contrast to other *F* factors, they are all equally susceptible to elimination by thymine deprivation. At 25 μg./ml., approximately 50% elimination of *F* factors occurred from all strains, including that carrying *F13*.

Scaife & Pekhov (1964) suggest that in W3747, the strain in which the *F13* factor arose, the chromosomal genes carried by the F prime are deleted from the bacterial chromosome. This conclusion is difficult to reconcile with the ability to produce F^- clones (by the criteria of insensitivity to male specific phage and inability to transfer lac^+) by thymine deprivation of a thymineless mutant of W3747.

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Appendix 3 (Cont'd)

If the rate of loss of plasmid DNA circles is proportional to the molecular weight of the plasmid studied, it is interesting that although the Col Ib plasmid is the same size as the F sex factor it is eliminated much less frequently. The Col Ib plasmid might determine the synthesis of an enzyme which is able to repair or ligase these single-strand interruptions, this plasmid confers protection to Col Ib^+ bacteria against damage by U.V. irradiation.

The elimination of certain R-factors has been reported by Pinney & Smith (1971), who also claim some R-factors are refractory to elimination by thymine deprivation.

Experimental Section 4

In this short experimental section the ability of sex factors to integrate into the bacterial chromosome is measured. The criteria for assessing the ability to integrate involve the phenomenon of integrative suppression (Nishimura et al, 1971) and the donor status of initiation revertants.

The temperature-sensitive mutation affecting the initiation of DNA replication, T46, was isolated in the CR34 sub-line. The mutant strain is referred to as CRT46 and has the following genotype: thr leu thi thyA ilv lac mal ts_{DNA} F^- (Hirota et al, 1968). The strain CRT46 was infected in turn with the transmissible plasmids listed in Table 5. All infections and subsequent incubation of the selective plates were performed at 30°. No difficulty was experienced in the infection of CRT46, but CRT46 donor strains are very difficult to maintain either on plates, stab cultures, slopes or frozen in Dimethyl Sulphoxide (DMSO) at -70°.

4-A Which transmissible plasmids are able to integrate into the E. coli chromosome?

The defective replication of CRT46 was not complemented by any of the transmissible plasmids, when the plasmid remained autonomous in the cytoplasm. The experimental protocol was essentially the same as that of Nishimura et al (1971). Broth cultures supplemented with thymine were grown at 30° with good seration. The CRT46 donor cultures were diluted and plated on nutrient agar plates supplemented with thymine and prewarmed to either 30° or 42°.

The plates were immediately incubated at 30° and 42° in fan-assisted incubators.

The viable counts obtained at 30° and 42° in these experiments are shown in Table 23. The survival ratio, i.e. the viable counts at 42°/viable count at 30°, is also shown since it is used by Nishimura et al (1971). However, it should not be taken to indicate absolute frequencies because it is impossible to estimate the number of cells per plate when the revertants grow up into distinguishable colonies. There could be considerable residual cell division in these mutant strains. The survival ratio serves merely as a crude indication of the integration events. It appears that all the F-like transmissible plasmids are capable of some degree of integrative suppression and, therefore, integration.

4-B The isolation of Hfr-type donor strains

The integratively suppressed revertants grow very slowly in liquid cultures, making it very difficult to prepare log phase broth cultures to test their fertility. The donor status of the revertants was assessed by the plate-mating technique described in Appendix 6C(iv). The incubation of all master-plates and replicas was performed at 42°. To save time and also ensure a good replica for the duplicate log phase plate donor cultures, master-plates were inoculated in triplicate. A simple illuminated, inclined triple-template was built for this purpose (see Appendix 8). The same apparatus can be used to read the results of the plate matings and match the fertile patches. Three recombinant classes per mating can be examined, and some idea of polarity obtained.

TABLE 23

The viable counts of CRT46 donor strains at 30° and 42°C

Sex factor	Viable Count/ml at 30°	Viable Count/ml at 42°	Ratio 42°C/ 30°C
None	4.4×10^8	8.8×10^2	2.0×10^{-6}
F [illegible]	2.1×10^8	1.3×10^4	6.3×10^{-5}
F'lac	1.8×10^8	3.6×10^5	2.0×10^{-3}
Col V2	3.9×10^8	1.1×10^4	2.8×10^{-5}
Col V3	4.2×10^8	1.0×10^4	2.3×10^{-5}
Col B1	1.9×10^8	5.0×10^3	2.6×10^{-5}
Col B2	2.8×10^8	5.1×10^3	1.8×10^{-5}
Col B3	1.1×10^8	1.3×10^4	1.1×10^{-4}
Col B4	4.1×10^8	4.2×10^3	1.1×10^{-5}
Col VB	1.1×10^8	2.0×10^3	1.8×10^{-5}
R1 drd 19	2.9×10^8	1.0×10^4	3.4×10^{-5}
R192 drd F7	1.5×10^8	8.0×10^3	4.1×10^{-5}
Col Ib drd	1.5×10^8	3.0×10^2	2.0×10^{-6}
R64 drd 11	2.2×10^8	4.6×10^2	2.1×10^{-6}
R144 drd 3	2.5×10^8	5.0×10^2	2.0×10^{-6}

All the F-like CRT46 donor strains produce temperature-insensitive revertants which are highly fertile and, so far as can be judged from the plate matings are Hfr-type donors, transferring their chromosomes in an oriented, sequential manner. Unfortunately, they are not particularly useful donor strains, since their doubling time in broth at 42° is between 60-110 minutes, with the result that they are rapidly overgrown by true ts^{+}_{DNA} revertants. Since integrative suppression has only been used as a tool to demonstrate the ability of plasmids to integrate, no detailed analysis of the Hfr donor types will be presented. However, all the sex factors which formed Hfr donor strains integrated in the same general region, between 64-74 minutes on the standard chromosome map of E. coli (Taylor, 1970). There is no apparent bias to the direction of transfer.

A plate mating analysis of 1,500 temperature-resistant revertants selected by incubating CRT46 Col Ib^{+} drd at 42° failed to yield any evidence of increased fertility and no polarity of chromosome transfer. Another 500 revertants were selected from CRT46 newly infected with Col Ib drd, these also were infertile. A further 1,000 each from CRT46 R64 drd 11 and R144 drd 3 showed the same result as Col Ib drd. It was concluded that, since there was no increase in the survival ratio of CRT46 in the presence of these three I-like plasmids, and also no evidence of polarity of transfer, the plasmids Col Ib drd, R64 drd 11 and R144 drd 3 are unable to confer integrative suppression and, in fact, probably do not integrate into the E. coli chromosome. Of course, it is possible that integration events may occur which do not result in Hfr type donors.

CHAPTER 6

THE TRANSFER OF CHROMOSOME BY Hfr DONORS

Section 1

The models for chromosome transfer

1-1 The requirement for metabolic activity

In Hfr X F⁻ crosses, the donor chromosome is transferred to the F⁻ recipient bacteria in a known genetic sequence (Jacob & Wollman, 1958). An extensive study of the metabolic requirements of the Hfr and F⁻ parents has been made to determine the role of each parent in chromosome transfer. This has revealed that the donor requires active metabolism to initiate chromosome transfer and the recipient requires energy to control the rate of chromosome transfer (Curtiss & Charamella, 1966; Curtiss, Charamella, Stallions & Mays, 1968).

1-2 The mechanism of chromosome transfer

A number of models have been proposed to explain the transfer of donor chromosome DNA during bacterial mating. An important feature considered in all the models is the requirement for DNA synthesis in either the donor or the recipient or both parents for successful chromosome transfer. The model of Bouck & Adelberg (1963), based on the results of Nagata (1963), proposed non-replicative transfer, that is, transfer which is independent of donor DNA replication. The model of Jacob & Brenner (1963), developed from their hypothesis of the replicon and its regulation, favoured replication-dependent chromosome transfer, effective pair formation between donor and recipient bacteria providing the contact stimulus to commence transfer.

The energy from the DNA replication would then propel the newly synthesized donor chromosome through the conjugation bridge into the $\underline{F^-}$ recipient. This model assumed that the transferred donor material is double-stranded DNA, one strand being newly synthesized in the donor during transfer.

Autoradiographic analysis of ^{3}H-labelled DNA transferred to recipients during conjugation, under different conditions of labelling, showed that the amount of label transferred to the zygote was compatible with the transfer of newly synthesized double-stranded DNA, provided that no DNA synthesis was occurring in the adenine-requiring recipient. However, if, as now seems probable, DNA synthesis did take place in the recipient, the results of Gross & Caro (1966) are also compatible with the transfer of a single pre-existing strand of donor DNA, with subsequent synthesis of a complementary strand in the zygote.

1-3 The role of the recipient in chromosome transfer

The data obtained in other experiments suggested an important role for the recipient during conjugal transfer (Bonhoeffer, 1966; Bonhoeffer, Hosselbarth & Lehmann, 1967; Freifelder, 1967; Ohki & Tomizawa, 1968). Where the recipient was thermosensitive with respect to DNA synthesis, $\underline{ts_{DNA}}$, virtually no recombinants were formed when the cross was performed at 42° (Bonhoeffer, 1966). This was taken to indicate a lack of transfer. It was therefore proposed that the donor DNA transferred was single-stranded, the complementary strand being synthesized in the recipient during mating, and that this essential synthesis was responsible for pulling the donor strand into the zygote.

There is a considerable volume of evidence to support the concept of single-stranded transfer (Bonhoeffer & Vielmetter, 1968; Cohen, Fisher, Curtiss & Adler, 1968a.b; Ihler & Rupp, 1969; Ohki & Tomizawa, 1968; Rupp & Ihler, 1968; Vapnek & Rupp, 1970; Vielmetter, Bonhoeffer & Schütte, 1968), as well as for the synthesis of the complementary strand in the recipient (Bresler, Lanzov & Lukjaniec-Blinkova, 1968; Cohen et al, 1968a.b; Eisenberg & Pardee, 1969; Vapnek & Rupp, 1970). The synthesis of DNA has in fact been shown to accompany genetic transfer during conjugation (Blinkova, Bresler & Lanzov, 1965; Freifelder, 1966; Herman & Forro, 1964; Ishibashi, 1966; Ptashne, 1965; Silver, 1963).

It turns out that the synthesis of the complementary strand by the recipient is not a prerequisite for chromosome transfer. The transfer of DNA to the Bonhoeffer $\underline{ts}_{\underline{DNA}}$ $\underline{F^-}$ recipient at the restrictive temperature has now been demonstrated (Moody & Lukin, 1970). The lesion in this particular $\underline{ts}_{\underline{DNA}}$ mutant causes degradation of the recipient DNA at 42° (Buttin & Wright, 1968), so that this degradation could be the reason for the lack of recombinants at 42°, if the incoming Hfr DNA is similarly degraded. The degradation of the $\underline{ts}_{\underline{DNA}}$ recipient DNA is directly brought about by the $\underline{rec^+B}$ nuclease which normally attacks double-stranded DNA at a single-strand break, but does not break down single-stranded DNA (Buttin & Wright, 1968). If this nuclease also degrades the incoming Hfr DNA, it follows that either this DNA is double-stranded or there must be some synthesis prior to degradation which produces

double-stranded regions. No recombinants are formed for donor genes transferred prior to the ts^{+}_{DNA} Hfr allele, but entry of this allele rescues markers transferred subsequent to it, as well as the wild type allele itself. Additional experiments with other ts_{DNA} recipients which do not degrade their DNA at 42° show clearly that transfer occurs normally and recombinants are formed when DNA synthesis in the recipient is inhibited (see Experimental Section 5).

Section 2

Hfr chromosome transfer

The direction of chromosome transfer is believed to be determined by the orientation of the inserted sex factor (Beckwith & Signer, 1966; Scaife & Gross, 1963). The Jacob & Brenner model for chromosome transfer (Jacob & Brenner, 1963; Jacob, Brenner & Cuzin, 1963) predicts that transfer is obligatorily linked with DNA synthesis in the donor strain, this transfer replication being controlled by the F sex factor.

Hfr donor strains, thermosensitive for DNA synthesis, sire recombinants at a normal frequency when mated at the restrictive temperature (Bonhoeffer, 1966). This might suggest that the donor chromosome can be transferred in the absence of DNA synthesis is not irreconcilable with the Jacob-Brenner hypothesis, which predicts that the transferred donor DNA should be double-stranded, one strand being newly synthesized during transfer, since the ts_{DNA} mutation in these strains affects bacterial DNA synthesis only. On the other hand, transfer by Hfr donors in the Jacob-Brenner model is hypothesized as F-mediated. In support of this, Bresler et al (1968), in their

experiments with $\underline{ts}_{DNA}$ mutants, were led to conclude that there was a special transfer replication system operating under the control of the sex factor; they could detect DNA synthesis associated with conjugation even when both Hfr and F⁻ strains were $\underline{ts}_{DNA}$ mutants. However, since repair synthesis does occur in these strains at 42° (Couch & Hanawalt, 1967), the results of Bresler et al (1968) cannot be accepted as proof of the existence of a F-directed transfer replication system.

The results of Ohki & Tomizawa (1968) and Vapnek & Rupp (1970) demonstrated that the transferred DNA is single-stranded and its complement is synthesized in the recipient. Vapnek & Rupp (1970) also show that a strand with the same polarity as the transferred strand is synthesized in the donor during or after transfer. The asymmetric strand distribution observed in their transfer experiments could be generated by a replication mechanism such as the rolling circle model proposed by Gilbert & Dressler (1968).

The demonstration of linkage between terminal markers and those near the origin of an Hfr donor led Fulton (1965) to propose a continuous transfer of DNA during mating, the transfer of DNA being in excess of the unit length of the circular genome. Additional evidence has been offered for the model of continuous chromosome transfer by Moody & Lukin (1970), who demonstrated linkage between the terminal marker, the integrated F factor and the proximal marker during late transfer by an Hfr strain. This was done under conditions where the recombinants prior to the entry of the terminal marker are presumed to be degraded by a $\underline{ts}_{DNA}$ recipient at 42°.

The continuous transfer of a genetic linkage map (Fulton, 1965; Moody & Lukin, 1970) is the precise genetic equivalent of a rolling circle model of DNA synthesis mediating transfer.

Experimental Section 5

Chromosome transfer by Hfr donor

5-A The requirement for DNA synthesis in the recipient strain during conjugation

A number of mutants, thermosensitive with respect to DNA synthesis, were obtained from Dr. H. Goldfine. The mutants were all isolated in the sub-line CR34 after ethyl methane sulphonate (EMS) treatment. All these mutant F^- strains were used as recipients in standard Hfr crosses at 37° and 42°. The donor strains used were HfrH, Hfr Cavalli and Hfr P13. Selection was made for thr^+leu^+ recombinants by incubating on supplemented minimal medium at 30°, and for ts^+_{DNA} recombinants on nutrient medium at 42°.

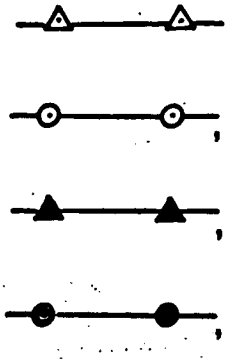
Some of the ts_{DNA} mutants behaved in the same way as Bonhoeffer's ts_{DNA} 43, no thr^+leu^+ recombinants being produced by HfrH in a cross at 42°, although both ts^+_{DNA} and thr^+leu^+ recombinants could be recovered in prolonged matings (140 minutes) as described by Moody & Lukin (1970). All the other ts_{DNA} mutants produced recombinants at the non-permissive temperature.

5-B The location of ts_{DNA} mutations determined by interrupted mating experiments

The crosses performed in 5-A indicated an approximate map location for the ts_{DNA} mutations. The positions of 15 of these

mutations were confirmed by performing interrupted mating experiments, using Hfr donors of opposite polarity to determine time of entry of the ts^{+}_{DNA} allele. Aliquots of the mating mixtures were interrupted as described by Moody & Lukin (1970) using a Low & Wood (1965) blending device. Results are shown in Figures 9-14. These map positions are in good agreement with the map locations of various other mutations affecting DNA synthesis (Figure 15). All four ts_{DNA} mutations including Bonhoeffer's ts_{DNA} 43, which prevented recombinant formation at 42^{o}, mapped together in Group B (Figure 15). The other mutations tested, which permit recombinant formation, were found to belong to Groups A and E. Although not all the groups of DNA mutants were examined and mapped in this study, it is reasonably safe to say that DNA synthesis in the recipient during mating is not essential for successful chromosome transfer.

Legend for Figures 9,10,11,12,13,14.



, thr leu strA

, ts_{DNA} strA

} recombinants selected in 37 cross.

, thrleu strA

, ts_{DNA} strA

} recombinants selected in 42 cross.

Figure 9.

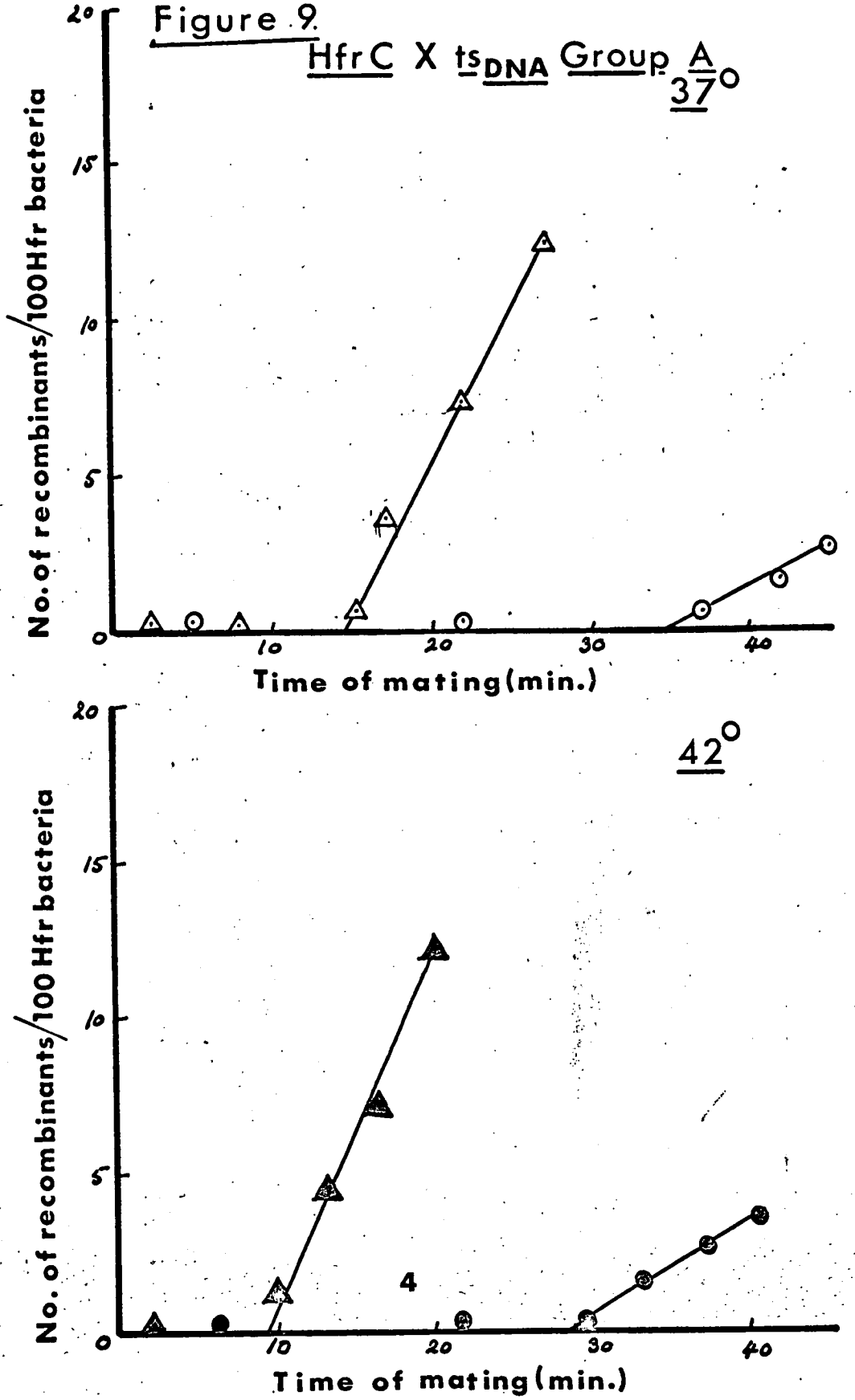

HfrC X ts DNA Group A
37°
No. of recombinants/100 Hfr bacteria
0
5
10
15
20
10
20
30
40
Time of mating (min.)
42°
No. of recombinants/100 Hfr bacteria
0
5
10
15
20
10
20
30
40
Time of mating (min.)
4

Figure 10

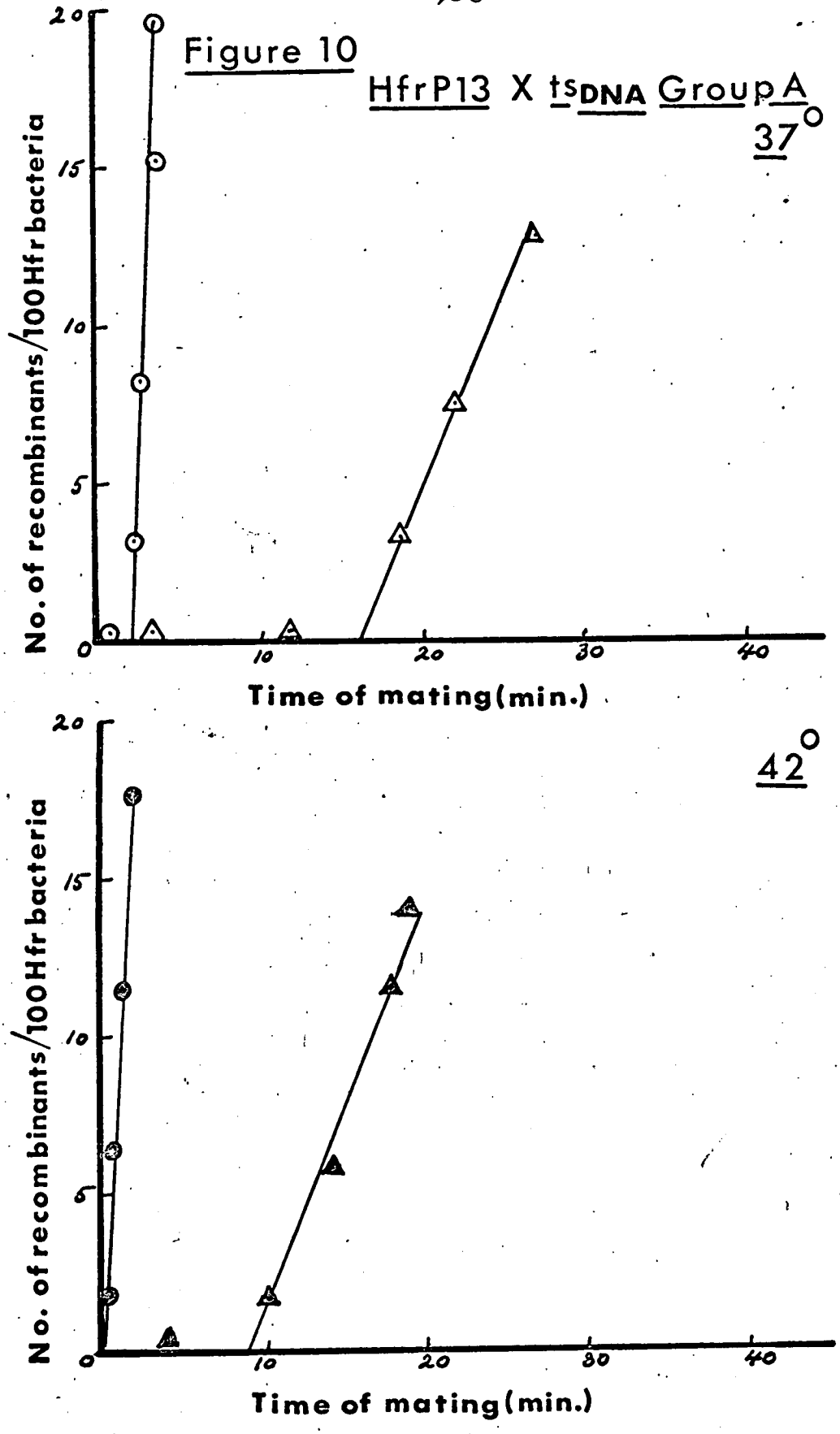

HfrP13 X tsDNA GroupA
37°
No. of recombinants/100Hfr bacteria
Time of mating(min.)
42°
No. of recombinants/100Hfr bacteria
Time of mating(min.)

Figure 11

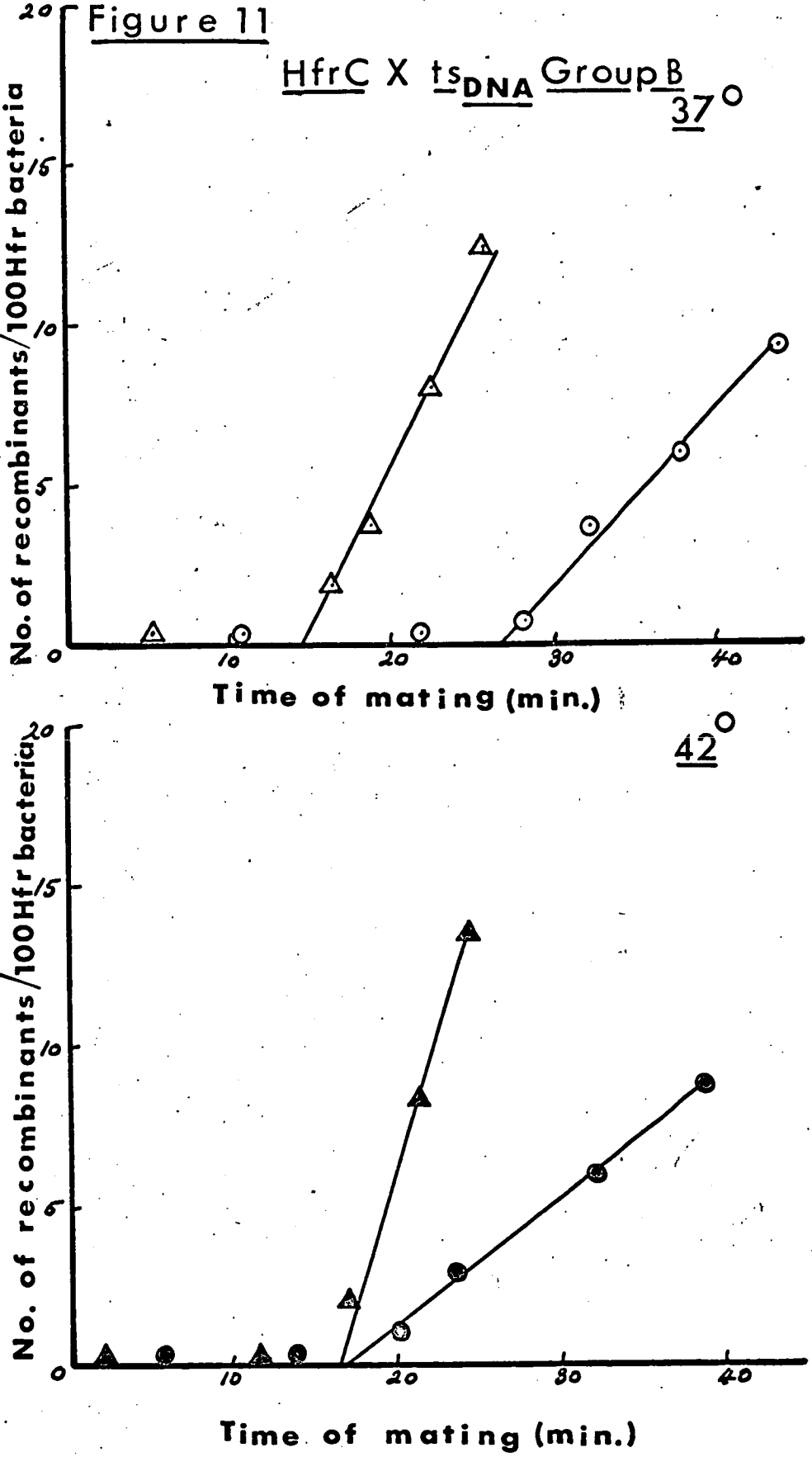
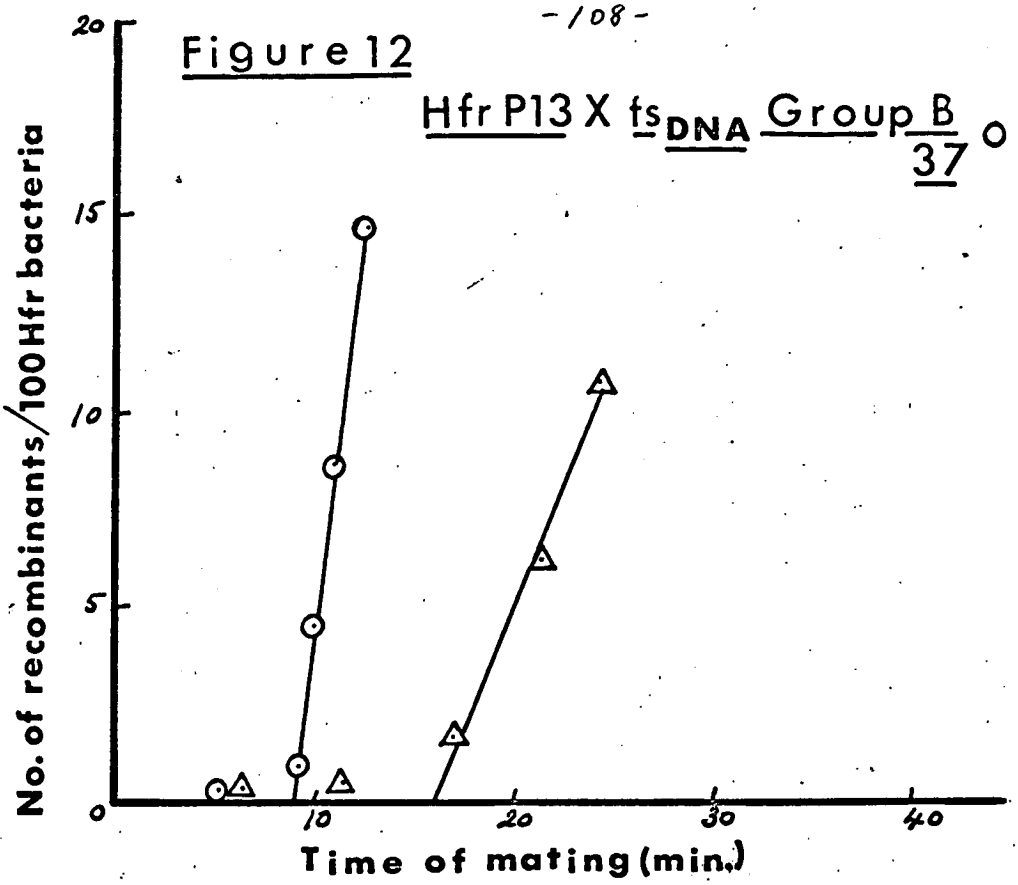

HfrC X ts DNA Group B
37°
No. of recombinants/100 Hfr bacteria
0
5
10
15
20
10
20
30
40
Time of mating (min.)
42°
No. of recombinants/100 Hfr bacteria
0
5
10
15
20
10
20
30
40
Time of mating (min.)

Figure 12


Hfr P13 X fs DNA Group B
37°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)
0
5
10
15
20
30
40

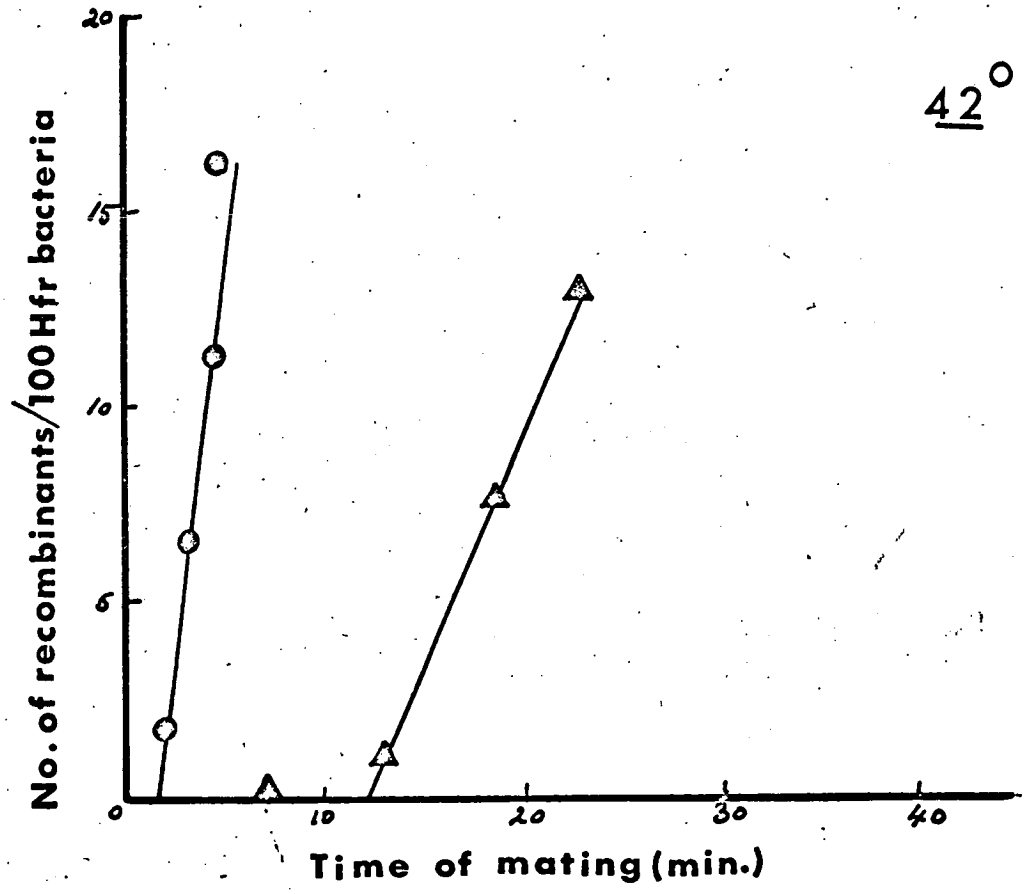

42°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)
0
6
10
15
20
30
40

Figure 13

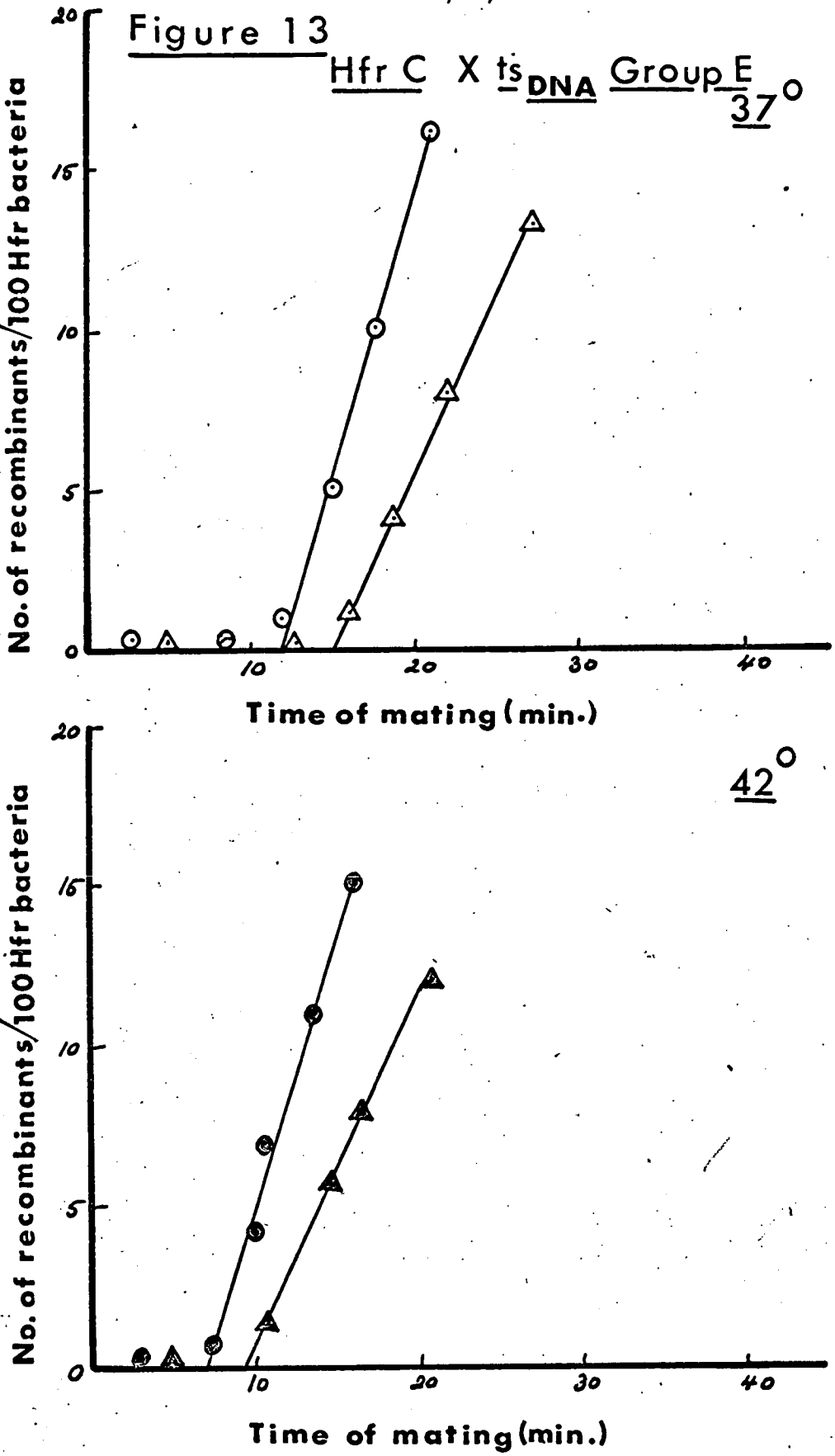

Hfr C X ts DNA Group E
37°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)
42°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)

Figure 14

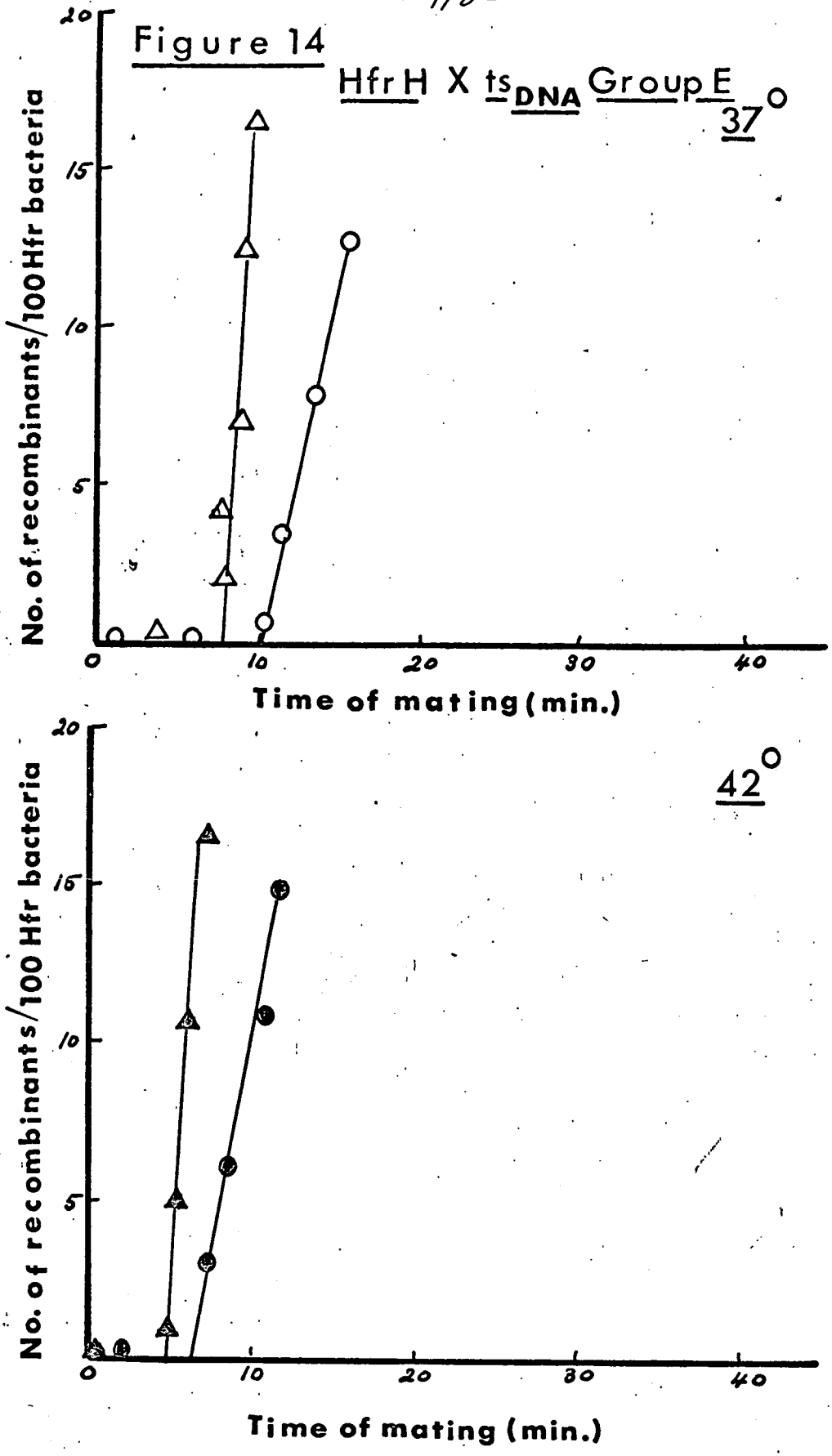
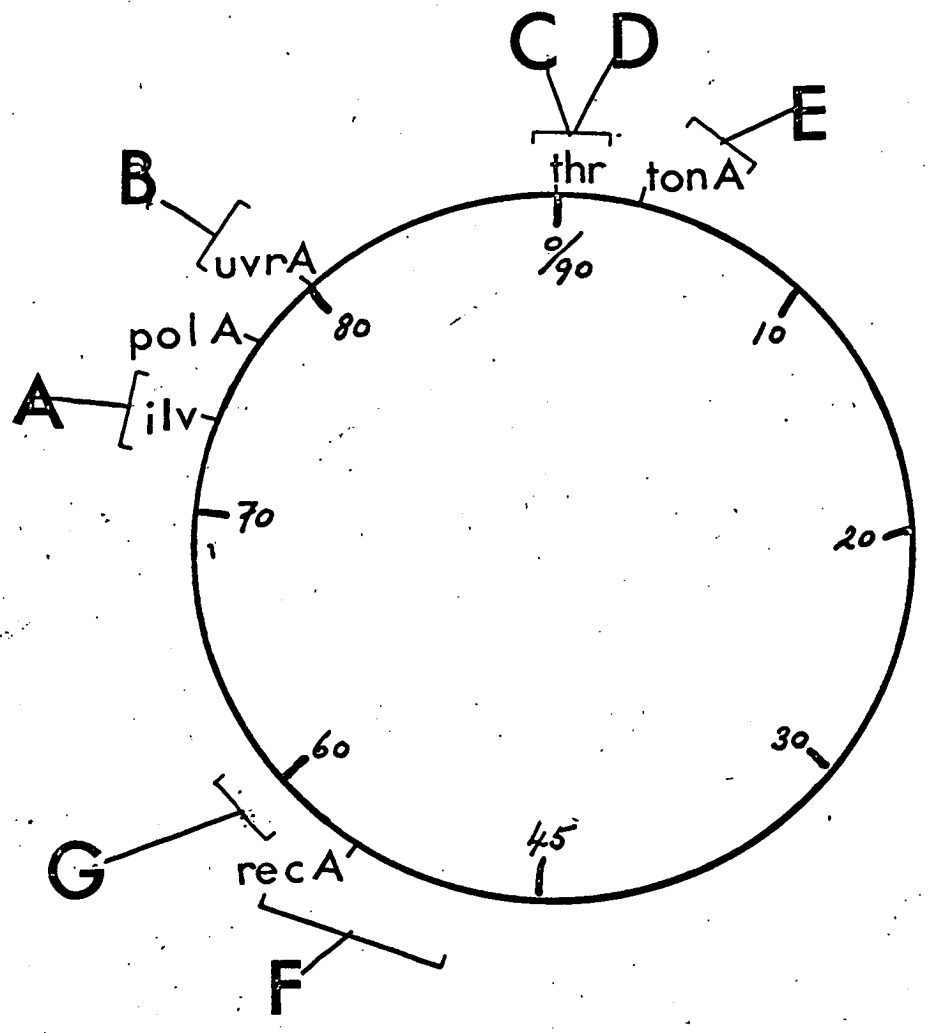

Hfr H X ts DNA Group E
37°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)
42°
No. of recombinants/100 Hfr bacteria
Time of mating (min.)

Figure 15.



The locations of some ts$_{DNA}$ mutations on the map of E.eoli K12.

after Gross (1971).

Appendix 4

Chromosome transfer during bacterial mating

E. E. M. Moody and A. Lukin

J. Mol. Biol. (1970) 48, 209-217.

Reprinted from *J. Mol. Biol.* (1970) **48**, 209–217

Chromosome Transfer during Bacterial Mating

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J. Mol. Biol. (1970) **48**, 209–217

Chromosome Transfer during Bacterial Mating

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(*Received 12 August 1969, and in revised form 11 November 1969*)

The transfer of Hfr chromosome to the Bonhoeffer ts_{DNA} female has been shown to occur normally at the restrictive temperature. The ts_{DNA} mutation has been mapped by both interrupted mating experiments and linkage analysis, and is located at about 82 minutes on the Taylor & Trotter (1967) map of *Escherichia coli*.

A possible mechanism of the protection of the ts_{DNA} female from thermal death by mating is discussed, and some evidence is presented supporting the model of continuous chromosome transfer (Fulton, 1965).

1. Introduction

In Hfr × F⁻ matings, the donor chromosome is transferred to the recipient in a known genetic sequence (Jacob & Wollman, 1958). Two models have been proposed to explain this transfer.

Model 1. DNA replication in the donor is required and provides the energy for transfer of DNA to the recipient. Therefore, only newly replicated DNA is transferred (Jacob & Brenner, 1963; Jacob, Brenner & Cuzin, 1963).

Model 2. Transfer occurs after replication has been completed. Therefore, transfer is independent of replication (Bouck & Adelberg, 1963).

The evidence for the models will be dealt with in the Discussion. However, the majority of experiments are interpreted as supporting the Jacob–Brenner hypothesis. Bonhoeffer (1966) cast doubt on the validity of this model. He showed that an Hfr strain, thermosensitive (*ts*) with respect to DNA synthesis, gave the normal yield of recombinants when mated at the restrictive temperature. The reciprocal cross, using a $ts_{DNA}F^-$ strain gave 500 times fewer recombinants at 42°C as compared to 37°C. He concluded that DNA synthesis in the recipient is required for effective transfer; one hypothesis to explain this phenomenon was that only one DNA strand is transferred from the donor, the complementary strand being simultaneously synthesized in the recipient during transfer.

Confirmatory evidence for the active role of the female was provided by Bonhoeffer, Hosselbarth & Lehmann (1967). They discovered that not only were recombinants not produced by a ts_{DNA} recipient at the restrictive temperature, but that these recipients failed to synthesize any β-galactosidase at a time when control experiments showed that the structural gene for this enzyme should have been transferred to the wild-type female.

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Evidence is presented here that the wild-type HfrH strain does in fact transfer its chromosome to the Bonhoeffer ts_{DNA} female at 42°C, since the mutation has been mapped at the restrictive temperature, using the time of entry of the wild-type ts^+ allele. The latter part of this paper offers support for the model of continuous chromosome transfer (Fulton, 1965).

2. Materials and Methods

TABLE 1

Substrains of Escherichia coli *used were as follows:*

Strain	Genotype†	Reference
CR34	*thr leu thy lac* $^-$ *str-r* F $^-$	Okada, Yanagisawa & Ryan, 1960
CR34	*thr leu thy lac* $^-$ *str-r spc-r* F $^-$	
CR34	*thr leu thy lac* $^-$ *str-r* ts_{DNA} F $^-$	Bonhoeffer, 1966
HfrH	*prototrophic* λ^-	Hayes, 1953
Hfr Reeves 1	*met*	Reeves, 1960
Hfr Cavalli	*met*	Cavalli-Sforza, 1950
Hfr Cavalli	*met ilv ampA*	Ericksson-Grennberg, 1968
Hfr P13	*cys his*	Hirota, Jacob, Ryter, Buttin & Nakai, 1968

† Symbols for genetic markers: *thr*, *leu*, *thy*, *met*, *ilv*, *cys* and *his*, requirement for threonine, leucine, thymine, methionine, isoleucine plus valine, cysteine and histidine respectively; *lac* $^-$, inability to utilize lactose as sole carbon source; *str-r*, resistance to streptomycin; *ampA*, resistance to ampicillin; *spc-r*, resistance to spectinomycin; ts_{DNA}, thermosensitive with respect to DNA synthesis.

(a) *Media*

All bacterial cultures and crosses were made in Oxoid no. 2 nutrient broth (25 g/l., pH 7·2). Viable counts and selection for ts^+_{DNA} recombinants were made on nutrient agar (Oxoid no. 2 broth, solidified with 1·5%, w/v, Davis New Zealand agar), results being read after overnight incubation at 37 or 42°C.

Recombinants for nutritional markers were scored on M9 (Adams, 1959) minimal–glucose–agar supplemented according to the required selection. Nutrient broth to a final concentration of 0·5% was added to M9 minimal–glucose–agar.

Thymine and amino acids were used at a final concentration 20 μg/ml., and streptomycin and ampicillin at 100 μg/ml. Dilutions were made in the standard phosphate buffer (pH 7·2) used in this laboratory. For each day's experiments cultures of thermosensitive strains were always set up in triplicate from three separate colonies, grown overnight with aeration at 35°C, plated for ts^+_{DNA} revertants and then stored at 4°C overnight. The culture with the fewest revertants was selected.

(b) *Mating conditions*

Overnight donor cultures were diluted 1/50 in fresh warm broth and grown with aeration to approx. 2 to 3 × 10^8 bacteria/ml. The selected overnight recipient culture (1 to 2 × 10^9/ml.) was diluted 1/10 at the beginning of an experiment. This dilution was always into fresh broth, prewarmed to 37 or 42°C. Donor : recipient mixtures were made in the ratio 1 : 10.

(c) *Interruption of mating*

0·1-ml. samples were removed from the mating mixtures, diluted and transferred to test tubes containing 2·5 ml. 0·6% w/v Difco agar. The tubes were then violently agitated for 10 sec on a blender of the type described by Low & Wood (1965). Samples were then plated by pouring the 2·5 ml. vol. of soft agar on to the surface of the selective medium and

allowing to set. Plates to be incubated at 42°C for selection of ts^{+}_{DNA} recombinants were kept on a thermostatically controlled hot-plate before and during plating.

3. Experiments and Results

(a) *Bacterial crosses*

The experiments described by Bonhoeffer (1966) were repeated using the donor strain Hfr Cavalli in addition to HfrH. The results of the HfrH × $ts_{DNA}F^{-}$ cross were in complete agreement with Bonhoeffer's results. There was a 500 to 1000 times lower yield of *thr*$^{+}$*leu*$^{+}$*str-r* recombinants when matings were carried out at 42°C, followed by incubation at 37°C after plating, compared to the number of recombinants from matings at 37°C, at all times up to 60 minutes after mixing when the matings were terminated.

TABLE 2

Yield of thr^{+}leu^{+}str-r *recombinants as function of time*

Donor	Recipient	Temperature (°C)	0	10	20	40	60 min
HfrH	CR34 F^{-}	37	0·001	0·02	5·0	12·0	16·0
		42	0·002	1·0	8·0	14·0	18·5
HfrH	CR34 $ts_{DNA}F^{-}$	37	0·002	0·02	5·0	10·5	14·0
		42	0·001	0·005	0·005	0·005	0·006
Hfr Cavalli	CR34 F^{-}	37	0·002	0·003	3·5	12·5	17·0
		42	0·002	0·002	5·0	15·0	19·0
Hfr Cavalli	CR34 $ts_{DNA}F^{-}$	37	0·003	0·004	3·0	12·0	16·5
		42	0·001	0·003	2·5	15·5	20·0

The yield is expressed in number of recombinants formed per 100 input donor bacteria.

Strain Hfr Cavalli, however, showed no reduction of *thr*$^{+}$*leu*$^{+}$*str-r* recombinants at 60 minutes when mated at 42°C. Indeed in many experiments more recombinants were actually produced in the 42°C mating. Transfer of the Hfr chromosome by this donor does, therefore, occur at the restrictive temperature for this female. In this case the appearance of *thr*$^{+}$*leu*$^{+}$*str-r* recombinants is almost certainly due to their rescue by transfer of the ts^{+}_{DNA} allele, since no *thr*$^{+}$*leu*$^{+}$*str-r* ts_{DNA} recombinants were found when crosses mated at 42°C were plated and incubated at 37°C.

(b) *Interrupted matings*

The map position of the ts_{DNA} mutation was first determined by interrupted mating experiments. The crosses with Hfr Cavalli had already indicated in which segment of the chromosome the ts_{DNA} mutation lay. Strains Hfr Cavalli and Hfr P13 were mainly used, although Hfr Reeves 1 was used in some early experiments. The matings were carried out at 37 or 42°C, *thr*$^{+}$*leu*$^{+}$*str-r* recombinants being selected at 37°C, and ts^{+}_{DNA}*str-r* recombinants at 42°C from both types of mating. The time of entry of the ts^{+}_{DNA} allele was determined using the above three Hfr strains in crosses at 37°C. The ts_{DNA} mutation is located at 80 to 82 minutes on the Taylor & Trotter (1967) map of *Escherichia coli*.

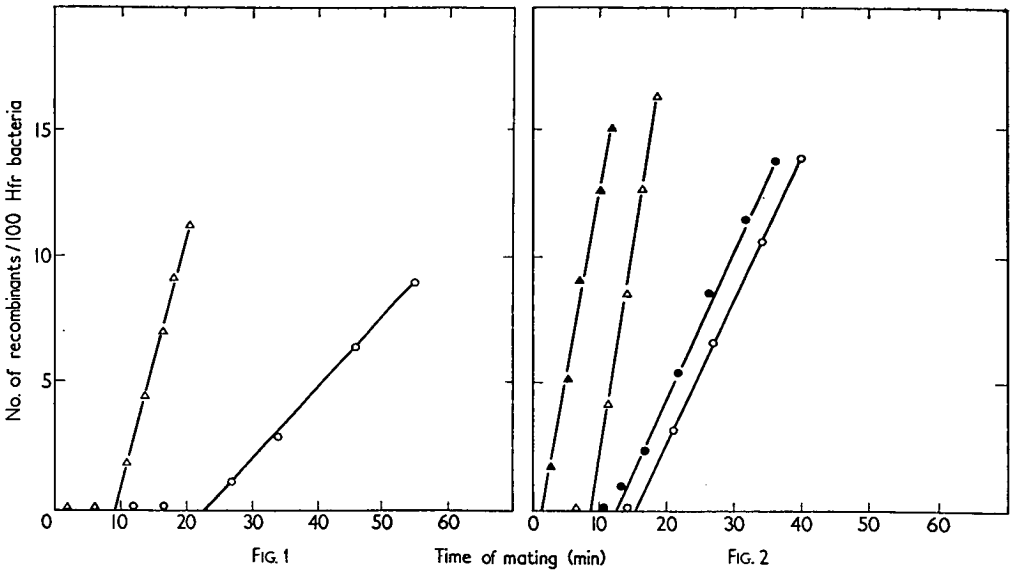

FIG. 1 FIG. 2

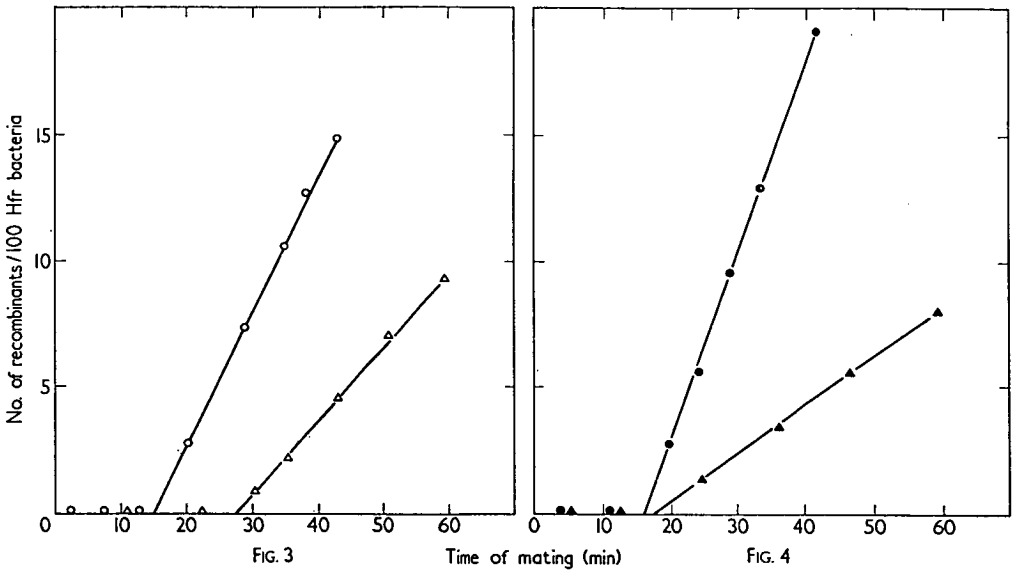

FIG. 3 FIG. 4

FIG. 1. Interrupted mating experiment selecting for *thr*$^+$*leu*$^+$*str-r* and for ts^+_{DNA}*str-r* recombinants. Donor strain Hfr Reeves 1 and the recipient is CR34 ts_{DNA}F$^-$; the mating was carried out at 37°C. The entry times of ts^+_{DNA} (—△—△—), and of the *thr*$^+$*leu*$^+$ (—○—○—) have been determined.

FIG. 2. Interrupted mating experiments with donor strain Hfr P13 × CR34 ts_{DNA}F$^-$; the matings were carried out at 37°C, selecting ts^+_{DNA}*str-r* (—△—△—) and *thr*$^+$*leu*$^+$*str-r* (—○—○—); and at 42°C, selecting ts^+_{DNA}*str-r* (—▲—▲—) and *thr*$^+$*leu*$^+$*str-r*(—●—●—).

FIG. 3. Interrupted mating experiment with donor strain Hfr Cavalli × CR34 ts_{DNA}F$^-$, selecting *thr*$^+$*leu*$^+$*str-r* (—○—○—), and ts^+_{DNA}*str-r* (—△—△—). The temperature of the mating was 37°C.

FIG. 4. Interrupted mating experiment with donor strain Hfr Cavalli × CR34 ts_{DNA}F$^-$, selecting *thr*$^+$*leu*$^+$*str-r* (—●—●—), and ts^+_{DNA}*str-r* (—▲—▲—). The temperature of the mating was 42°C.

It was noted that in interrupted mating experiments with Hfr Cavalli at 42°C, there was a delay in the apparent time of entry of *thr*$^+$*leu*$^+$, as compared with mating at 37°C, which now coincided with the time of entry of ts^+_{DNA}. These two markers had quite separate entry times in the crosses made at 42°C with Hfr strains of opposite polarity to that of Hfr Cavalli. In general, markers appeared earlier in matings at 42°C than in those at 37°C. Wood (1968) has reported that transfer velocity in Hfr × F$^-$ matings approximately doubles between 38 and 42°C, in contrast to population growth rate and rate of DNA synthesis, which drop significantly.

(c) *Map position by linkage analysis*

An attempt to determine the map position of the ts_{DNA} locus by phage P1 transduction was abandoned because the ts_{DNA} recipient proved to be very inefficient both for making lysates and for transduction.

TABLE 3

Linkage between ts_{DNA} *and* ampA

Donor	Recipient	Recombinant class selected	Unselected marker§
Hfr Cavalli *ampA*	CR34 ts_{DNA}	†ts^+_{DNA} *str-r*	*ampA* 100%
Hfr Cavalli *ampA*	CR34 ts_{DNA}	‡*ampA str-r*	ts^+_{DNA} 98%

Recombinants from Hfr Cavalli *ampA* × CR34 ts_{DNA}F$^-$; time of mating was 60 min, temperature of mating was 37°C.

† ts^+_{DNA} selected on nutrient agar with thymine + streptomycin, incubated at 42°C overnight.

‡ Sample held at 37°C for 4 hr for expression of *ampA* before plating on nutrient agar + thymine + ampicillin.

§ 100 recombinants were purified on the same medium on which they were selected; the percentage of the unselected markers was determined by replica plating from the purified recombinants.

Confirmation of the map position was obtained in crosses between Hfr Cavalli *ampA* × ts_{DNA}F$^-$, selection being made for ts^+_{DNA}*str-r* and for *ampA str-r* recombinants. The purified recombinants were scored for *ampA* and ts^+_{DNA} respectively. This analysis (Table 3) shows that the two markers are very closely linked and confirms a map position of about 82 minutes (Ericksson-Grennberg, 1968).

(d) *Protection by mating*

It was observed on checking viable counts before and after matings at 42°C, that the ts_{DNA}F$^-$ bacteria in the mating mixtures were not killed to anything like the extent as in an unmated control. Survival curves for the ts_{DNA}F$^-$ bacteria were determined in mating mixtures at 42°C with HfrH and Hfr Cavalli; an unmated ts_{DNA}F$^-$ culture was the control.

The unmated female bacteria died exponentially at the restrictive temperature. The viable counts of the ts_{DNA}F$^-$ bacteria in the mating mixtures initially dropped to about 10% survival, and then remained constant up to 60 minutes, when the experiment was terminated. The survival level of the ts_{DNA} bacteria seemed to be determined by the proportion of Hfr cells in the mixture. Since this protective effect occurs with

HfrH at times up to 60 minutes, it is unlikely to have anything to do with the transfer of the wild-type allele. This was checked. Survivors at 37°C from the HfrH × $ts_{DNA}F^-$ mating at 42°C were replica-plated to new plates and incubated at 42°C. They were all temperature sensitive.

(e) *Chromosome transfer by strain HfrH*

Crosses at 42°C with Hfr strains other than HfrH have shown that chromosome transfer to $ts_{DNA}F^-$ bacteria occurs at the restrictive temperature. It was decided to reinvestigate the HfrH × $ts_{DNA}F^-$ cross at 42°C, but extending the mating time to 140 minutes, selecting for both ts^+_{DNA} and *thr*$^+$*leu*$^+$ recombinants.

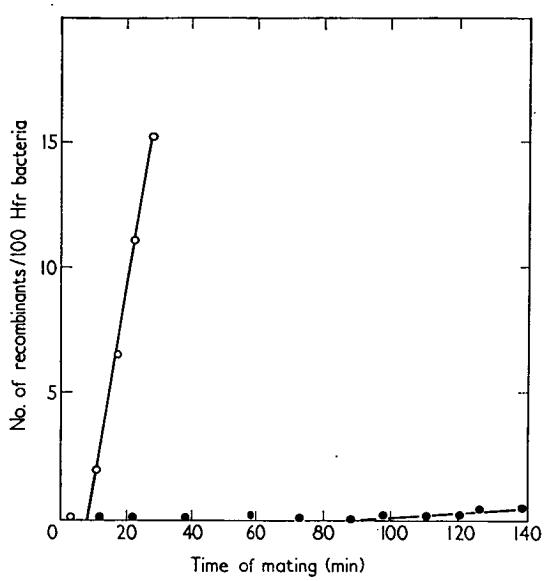

FIG. 5. The kinetics of recombinant formation in a cross HfrH × CR34 $ts_{DNA}F^-$, selecting for *thr*$^+$*leu*$^+$*str-r* recombinants in matings performed at 37°C (—○—○—), and at 42°C (—●—●—).

Two new and interesting features of this cross emerged from these experiments. The first, and most relevant to the Bonhoeffer effect, was that approximately the same number of ts^+_{DNA}*str-r* recombinants arose on streptomycin–nutrient agar, irrespective of whether mating and subsequent incubation were at 42°C, or mating carried out at 37°C and the plates incubated at 42°C. Interrupted mating experiments conducted at 42°C showed that the ts^+_{DNA} locus was transferred to the ts_{DNA} recipients at about 80 minutes after the beginning of mating, that is, at about the same time as at 37°C. It is therefore evident that the HfrH chromosome is transferred normally to ts_{DNA} recipient bacteria at 42°C, despite the fact that recombinants for proximal loci appear at only an insignificant frequency.

In the case of selection for *thr*$^+$*leu*$^+$ recombinants, the results obtained were the same as in the previous experiments for times up to 80 minutes. Transfer of *thr*$^+$*leu*$^+$ occurred at a low level, yielding about 0·005% recombinants.

The second interesting feature of this cross was that a secondary increase in the number of *thr*$^+$*leu*$^+$ recombinants commenced at about 90 minutes after the beginning

of mating and continued up to termination of mating at 140 minutes. This result suggested two possibilities. One is that transfer of the ts^{+}_{DNA} locus was occurring after about 80 minutes of mating, with the formation of ts^{+}_{DNA} recombinants. These $ts^{+}_{DNA}F^{-}$ bacteria could have mated anew with Hfr bacteria and the *thr*$^{+}$*leu*$^{+}$ loci have begun to be transferred at the normal time, i.e. about ten minutes later, which would correspond to 90 minutes in the original mating mixture. The other possibility was that we were observing an example of the continuous chromosome transfer described by Fulton (1965). Experiments were designed to distinguish between these two possibilities.

(f) *Remating or continuous transfer*

Strain HfrH was mated with the Bonhoeffer $ts_{DNA}F^{-}$ strain at 42°C for 140 minutes; samples of the mixture were taken at 10-minute intervals, blended and plated for *thr*$^{+}$*leu*$^{+}$$ts^{+}_{DNA}$*str-r* recombinants. No recombinants of this type appeared at the dilutions plated until after 90 minutes of mating. The frequency of such recombinants was at best 0·5% of input Hfr bacteria at 140 minutes.

These recombinants were purified and checked that they were indeed *thr*$^{+}$*leu*$^{+}$ ts^{+}_{DNA}*str-r*. Maleness was tested by cross streaking against the male-specific bacteriophage MS2 (Davis, Strauss & Sinsheimer, 1961). This showed that 59 recombinants out of 60 were MS2-sensitive and, therefore, males. These male strains were then tested for the Hfr state by cross streaking against a *thr leu* spectinomycin-resistant (*spc-r*) F^{-} strain, selecting for early transfer of *thr*$^{+}$*leu*$^{+}$. Spectinomycin was used as the contraselective agent, since these recombinant males are streptomycin resistant. All these recombinant males seemed to be Hfr in this crude test. Five of them were finally checked by a standard cross, and all were found to give at least 5% *thr*$^{+}$*leu*$^{+}$ recombinants with the spectinomycin-resistant recipient. The interpretation of these results will be dealt with in the Discussion.

4. Discussion

A number of models have been proposed to explain the transfer of donor DNA to recipient cells during mating. The model of Bouck & Adelberg (1963) based on the results of Nagata (1963) proposed non-replicative transfer. The second model is that of Jacob & Brenner (1963) developed from their hypothesis of the replicon and its regulation, where transfer is dependent upon replication. Cell contact during conjugation provides the stimulus to commence replication transfer. This second model should now be divided into two models: double-stranded transfer (Jacob & Brenner, 1963), one of the strands being newly synthesized in the donor during transfer, and single-stranded transfer (Cohen, Fisher, Curtiss & Adler, 1968), where the complementary strand is synthesized in the recipient (Ohki & Tomizawa, 1968).

The results of Bonhoeffer (1966) questioned the Jacob–Brenner hypothesis. He showed that the yield of recombinants, sired by an Hfr strain, thermo-sensitive with respect to DNA synthesis (ts_{DNA}), was normal when mating was carried out at the restrictive temperature. On the contrary, in the reciprocal cross, where the female was ts_{DNA}, virtually no recombinants were formed at 42°C. Bonhoeffer (1966) interpreted these results as indicating lack of transfer. It was proposed that the donor DNA transferred in mating was single stranded, and that the complementary strand was synthesized in the female, this synthesis being required for transfer. Clark & Margulies

(1965) demonstrated that lack of recombinants is no criterion for lack of transfer, since the formation of recombinants involves other mechanisms in addition to transfer. However, Bonhoeffer *et al.* (1967) showed that not only were no *lac*$^+$ recombinants formed in matings with a ts_{DNA} recipient at 42°C, but that no β-galactosidase synthesis followed the mating, in contrast to control matings at 37°C. They interpreted this absence of gene expression, which is not dependent on recombinant formation, as good evidence for lack of transfer.

In this paper we have presented results which invalidate the role of the female suggested by Bonhoeffer's experiments. Transfer to the ts_{DNA} female has been demonstrated to occur normally at the restrictive temperature. It has been shown that the thermo-sensitivity of the ts_{DNA} female breaks down its own DNA at 42°C (Buttin & Wright, 1968; Moody, unpublished data). The degradative enzyme appears to be the product of the *recB* gene (Buttin & Wright, 1968). This degradation of DNA in the ts_{DNA}F$^-$ strain could be the reason why no recombinants are formed in matings at 42°C. The postulated small regions of homology between the proximal extremity of the donor chromosome and the recipient chromosome, required for successful recombinant formation (Curtiss, Charamella, Stallions & Mays, 1968), could be destroyed, or the incoming donor DNA could be generally degraded, as the absence of gene expression at the restrictive temperature suggests. The protection from thermal death by mating might also be taken to indicate that the latter explanation is true. The increased transfer velocity observed at 42°C (Wood, 1968) might provide sufficient substrate to saturate the breakdown enzyme and prevent degradation of the F$^-$ DNA, as has been suggested to happen in host restriction (Glover & Colson, 1965). This explanation cannot be quite so simple, however, since although the introduction of a *recB* mutation into Bonhoeffer's ts_{DNA}F$^-$ strain prevents DNA degradation, as measured by release of trichloroacetic acid-soluble material, it does not prevent loss of viability (Buttin & Wright, 1968).

The question of whether single- or double-stranded material is transferred remains open. The experiments of Gross & Caro (1966) strongly support double-stranded transfer, as predicted by the Jacob–Brenner model. Nevertheless some experiments by other workers favour single-stranded transfer (Bonhoeffer, 1966; Cohen, Allision, Adler & Curtiss, 1967; Vielmetter, Bonhoeffer & Schutte, 1968). Single-stranded DNA has been isolated from females after mating (Cohen *et al.*, 1968), but these workers keep in mind the possibility of double-stranded DNA being transferred and converted to single-stranded DNA in the female (see Piekarowicz & Kunicki-Goldfinger, 1968).

Our evidence in favour of Fulton's (1965) model of continuous transfer is based on the observation that virtually all the *thr*$^+$*leu*$^+$$ts^+_{DNA}$ recombinants formed after 90 minutes mating between HfrH and the ts_{DNA} female are Hfr. We propose that most of the DNA transferred, before entry of the ts^+_{DNA} locus, is destroyed in matings at 42°C; there is certainly no significant number of recombinants formed at shorter periods of mating. The linkage observed between ts^+_{DNA}, the integrated sex-factor and *thr*$^+$*leu*$^+$ can only be explained if they are all transferred on a continuous structure.

The question of the role of DNA synthesis in the female has not been answered in the data presented here. However, preliminary experiments with about 20 mutants, thermo-sensitive as regards DNA synthesis, show no reduction in the number of recombinants when mated at 42°C (Moody, unpublished data). The data presented we interpret as favouring the Jacob–Brenner hypothesis (1963).

We thank Dr F. Bonhoeffer, Dr Y. Hirota and Professor H. Boman for the bacterial strains they generously provided. We are grateful to Professor W. Hayes for stimulating discussion during the course of this work, and during the preparation of this manuscript.

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CHAPTER 7

CONCLUSIONS

Many transmissible plasmids are capable of transferring donor chromosomal material during conjugation. This chromosome transfer is believed to result from the interaction of the plasmids with the bacterial chromosome to form some type of physical association. The data obtained by Clowes & Moody (1966), and the work now reported in Experimental Section 1, suggests that the greater part of chromosome transfer by the majority of plasmids is dependent on the bacterial recombination, (REC) system. Virtually all transfer of donor chromosome is abolished when the donor strain is recombination-deficient, through a defect in the recA gene. Transfer is only partially affected by the presence of a recB mutation in the donor.

In the case of the majority of plasmids, these plasmid chromosome interactions, although perfectly adequate for chromosome transfer rarely result in a reciprocal genetic exchange to form an Hfr type donor. Indeed, the formation of stable Hfr clones does not account for more than 10-15% of the observed fertility of F^+ donor cultures (Broda, 1967, pers. commun.; Curtiss, 1968; Curtiss & Renshaw, 1965, 1969). Integration events are perhaps initiated but not often completed.

Chromosome transfer by the plasmids Col Ib drd and R1 drd 19 probably occurs by a mechanism which is independent of the bacterial REC system since it is unaffected by the presence of the recA mutation in the donor strain.

The transfer of chromosomal material by autonomous plasmids can be stimulated by small doses of ultraviolet light (Evenchik et al, 1969; Hayes, 1952a, 1953b; Rajchert-Trzpil & Dobrzański, 1968) (see Experimental Section 2). In a redefinition of the phenomenon of U.V.-induced donors (Evenchik et al (1969) proposed that the increased interaction of the F factor with the chromosome was due to the excision of a U.V. damaged strand of chromosomal DNA. If this excision occurs in a region of sex factor homology, this might result in greatly increased efficiency of pairing of the sex factor with the chromosome, perhaps forming a temporary structure capable of transferring chromosomal material. This is supported by the fact that the U.V. stimulation does not occur in uvr⁻ strains, where the excision of U.V. damage is defective, nor in rec⁻ strains.

Donor strains harbouring newly transferred transmissible plasmids also show a stimulation of chromosome transfer, and in these donor strains there is no reason to suppose that there is damage which leads to excision. Another objection to the general applicability of the Evenchik et al (1969) model can be found in the data obtained with the plasmid Col Ib drd. This plasmid normally transfers chromosomal genes at a very low frequency. The conventional interpretation of this would suggest that the Col Ib drd plasmid lacked genetic homology with the chromosome. However, the transfer of chromosome by Col Ib drd donor strains can be stimulated about 100-fold by both U.V. irradiation and new infection. It is unlikely that either treatment can increase to such an extent the affinity that the plasmid has for the chromosome. It is more reasonable

to propose that the stimulation of chromosome transfer is due to the induction of some plasmid function which promotes interaction with the bacterial chromosome. At the same time there is an apparent derepression of other Col Ib drd plasmid functions. This can be observed by an increased number of cells commencing the lethal synthesis of colicin, as judged by the numbers of lacunae.

Cells harbouring Col Ib are more resistant to U.V. damage than Col$^-$ bacteria (Howarth, 1965; Takano, 1966; Walsh & Meynell, 1967). Bacteria newly infected with Col Ib drd show a great increase in this U.V. resistance, which can even protect a newly infected recA strain from death following large U.V. doses. Under these same conditions there is a partial restoration of the recombination proficiency of these recA bacteria, measured by their ability, as recipients, to produce recombinants in an Hfr cross. Moreover, when either rec$^+$ or recA bacteria are newly with a Col Ib drd plasmid, these donors yield 100 times more recombinants than stably infected cells. The low level of residual transfer from all recA donors can also be markedly stimulated by the process of new infection by any plasmid, with the single exception of R1 drd 19 which already has a high transfer frequency. These data are perhaps consistent with the following model.

A model for the transfer of chromosome by autonomous transmissible plasmids

It is proposed that all transmissible plasmids can specify the synthesis of an endonuclease, which can make single-strand excisions in both the plasmid and chromosomal DNA. The synthesis of this

enzyme is normally repressed but, like the expression of the conjugal fertility systems of many Col or R-factors, or the synthesis of colicin by all Col factors, the repression occasionally breaks down, thus permitting the plasmid to interact with the chromosome.

The endonuclease activity of the plasmid R1 drd 19 is probably naturally derepressed, just as the F factor is a naturally occurring fertility-derepressed plasmid. The U.V. stimulation of donors and the stimulation observed in newly infected donor strains could thus be due to the derepression of this endonuclease system, similar to the U.V. induction or the zygotic induction observed with a λ lysogen.

The single-strand scissions produced in the plasmid chromosomal DNA by this endonucleolytic action are sites which can then be attacked by an exonuclease, possibly the rec$^+$B rec$^+$C nuclease, which would partially digest both of the cut strands in the 3'→5' direction. The extent of this digestion is controlled by the rec$^+$A product. In this way, the attempt at interaction in a recA donor might itself be a lethal event, both the chromosome and the sex factor becoming degraded, following endonuclease "nicking", so that chromosome transfer cannot occur.

Plasmids like Col Ib drd and R1 drd 19 which are unaffected in their ability to transfer chromosome by the recA mutation must be able to control the nuclease action in the absence of the rec$^+$A product, and it has been shown (Table 20) that the Col Ib drd factor at least can restore some degree of recombination function to recA recipient cells.

The rec$^+$B rec$^+$C exonuclease digestion exposes single stranded regions on both the plasmid and the chromosome and these regions will almost certainly be capable of some limited pairing. Hydrogen bonding in this region would give some stability to the structure. A repair-type DNA synthesis could then replace the digested strand of the plasmid using the exposed 3' end as a primer and the intact complementary strand as a template (see Figure 16). The plasmid endonuclease now cleaves the intact strand of the chromosome. The newly synthesized sex-factor strand could finally be joined to the chromosomal strand by a polynucleotide ligase, thus forming a continuous single-stranded structure from part of the sex-factor and the chromosome. Moreover, this structure preserves the 5'→3' continuity which has been reported for the transfer of DNA from Hfr (Rupp & Ihler, 1968) and from F$^+$ donors (Vapnek & Rupp, 1970). The transferred material would be single-stranded and there is no requirement for DNA synthesis, apart from some repair-type synthesis.

This structure, shown in Figure 16(5), is therefore capable of transferring donor chromosomal genes to a recipient and its formation is REC-dependent for most plasmids. However, it can never lead to a stable integration event, because certain regions required for a reciprocal genetic exchange are lost through exonuclease digestion.

The formation of Hfr type (see Figure 17) may involve the plasmid endonuclease making single-strand scissions. If this is the case, it is more likely that there is an unwinding of the cleaved strands rather than digestion by an exonuclease. The plasmid-

Figure 16.

A model for chromosome transfer by autonomous plasmids.

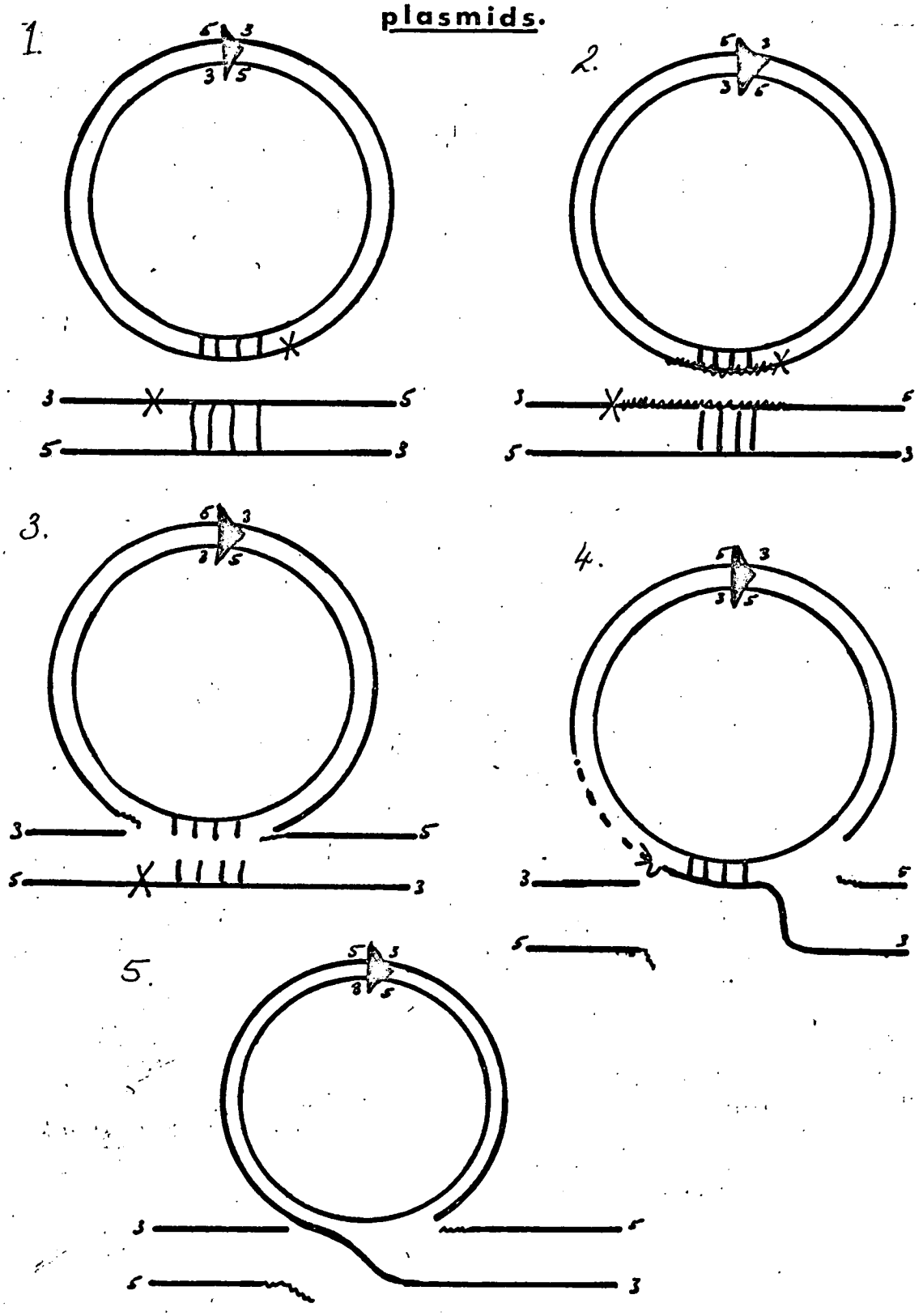
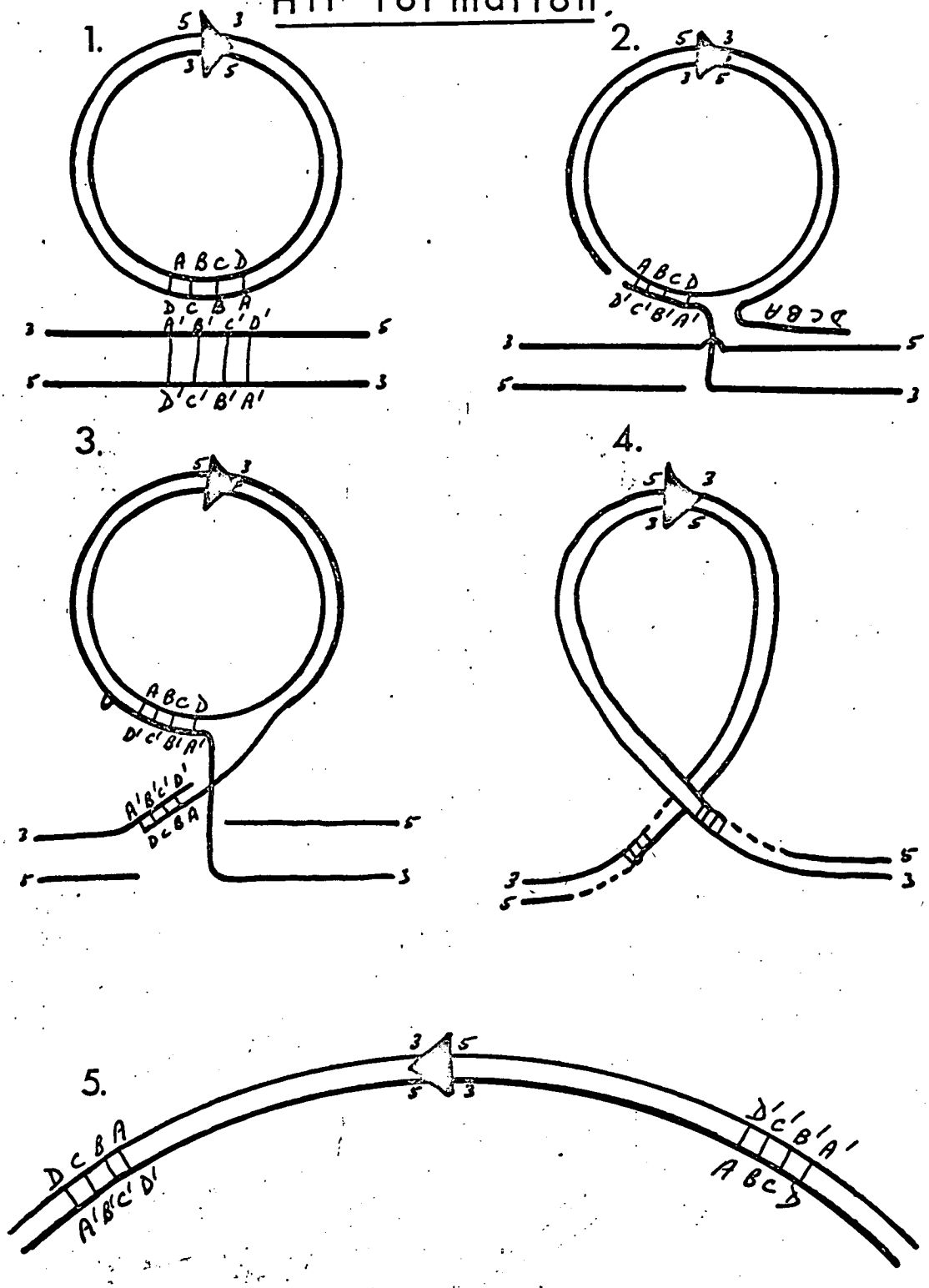


Figure 17.

Hfr formation.



chromosome complex probably is stabilized by hydrogen-bonding until a limited repair synthesis and ligase action joins one strand of the plasmid with one of the chromosomal strands. After this fairly stable structure is formed, a progression of endonuclease action, followed by hydrogen bonding, repair synthesis and ligase may finally produce the structure shown in Figure 17(5).

Appendix 5

A. Media

Nutrient Broth:

Oxoid No. 2 nutrient broth powder 25g;

distilled water to 1 litre, pH = 7.2

EMB peptone base:

Difco Bacto Casamino acids 42.4g;

Difco Bacto Yeast extract 5.2g; NaCl 27g; $K_2H\ PO_4$ 10.4g;

distilled water to 1 litre

Minimal Salts (X4):

NH_4Cl 20g; $NH_4\ NO_3$ 4g; anhydrous $Na_2\ SO_4$ 8g;

anhydrous $K_2\ HPO_4$ 12g; $KH_2\ PO_4$ 4g; $Mg\ SO_4\ 7H_2O$ 0.4g;

distilled water to 1 litre

Minimal medium:

Minimal salts (X4) diluted $\frac{1}{4}$ with distilled water.

Glucose added to 0.2%

Phosphate buffer:

Anhydrous $Na_2\ HPO_4$ 7g; $KH_2\ PO_4$ 3g; NaCl 4g; $Mg\ SO_4$

$7H_2O$ 0.2g; distilled water to 1 litre

Nutrient Agar:

Oxoid nutrient broth solidified with 15.0g./l. of Davis

New Zealand Agar

Water Agar:

Davis New Zealand agar 20g; distilled water to 1 litre,

adjust pH = 7.2

Minimal agar:

Minimal salts (X4) diluted $\frac{1}{4}$ with water agar.

Glucose added to 0.2%

Appendix 5 (Cont'd)

Water Soft agar:

Difco Bacto agar 7g; distilled water to 1 litre

EMB dyes:

Eosin yellow 4% (w/v); methylene blue 0.65% (w/v)

EMB (Sugar) agar:

300 ml water agar; 75 ml EMB peptone base; 4 ml Eosin solution; 4 ml methylene blue solution; 20 ml of a 20% Sugar solution

EMB-O agar:

500 ml nutrient agar; 4 ml Eosin solution; 4 ml methylene blue solution

Amino acids:

Made up as a solution of 4 mg./ml. and used at a final concentration of 20 µg/ml.

Vitamin B1:

Thiamin hydrochloride, used at a final concentration of 1 µg/ml.

Thymine:

Used at a final concentration of 50 µg/ml.

Sugar Solution:

Made up as 20% solutions and used at a final concentration of 0.2%, except in EMB sugar plates, where the final concentration is 1.0%

Appendix 5 (Cont'd)

B. Antibiotics

Penicillin:

Benzyl penicillin (Glaxo) used at a final concentration of 20 µg/ml.

Tetracycline:

Achromycin hydrochloride (Cyanamid) was used at a final concentration of 50 µg/ml.

Streptomycin:

Streptomycin sulphate BP (Glaxo) used at a final concentration of 200 µg/ml. for the contraselection of donors, with chromosomal markers. At a final concentration of 20 µg/ml. to select an R-factor resistance. However, to select against a donor strain harbouring a drug resistance for streptomycin, the final concentration was 5,000 µg/ml.

Spectinomycin:

Spectinomycin base, a gift from the Upjohn Chemical Company, was used at a final concentration of 100 µg/ml. for both the contraselection with chromosomal markers and the selection of an R-factor resistance.

Kanamycin:

Kanamycin sulphate BPC (Bayer) used at a final concentration of 20 µg/ml.

Chloramphenicol:

Chloromycetin (Parke Davis) used at a final concentration of 20 µg/ml.

Appendix 5 (Cont'd)

Nalidixic Acid:

As sodium nalidixate, used at a final concentration of 40 µg/ml.

Multodisks:

Oxoid multodisks, Code No. 30-19N, carrying the following antibiotics impregnated on paper:- tetracycline, streptomycin, sulphonamide, kanamycin, chloramphenicol, ampicillin.

Appendix 6

A. Bacteria

All bacterial strains were maintained in Difco agar stabs at room temperature. In addition to the stab cultures certain stocks were maintained frozen in Dimethyl Sulphoxide DMSO. Bacterial strains in current use for experimental work were also maintained on nutrient agar plates at 4°.

(i) Difco stabs — Difco Bacto nutrient broth 9g; Difco Bacto agar 7.5g; NaCl 5g; thymine 0.1g; distilled water to 1 litre

(ii) DMSO cultures — Concentrate 250 ml log broth cultures by filtration through Millipore filters. Resuspend the filters in 10 ml oxoid broth and add 0.8 ml DMSO. Store at -70°.

B. Culture conditions

Overnight cultures were prepared by innoculating 5 ml of oxoid broth, in a screw-capped bottle, with a single colony of the desired strain, followed by a 12-18 hr. incubation period usually at 37° without aeration. Cultures prepared this way normally have a viable count of between 4.0-8.0 x 10^8/ml. Log phase cultures were prepared by diluting the overnight broth cultures $^1/_{50}$ in fresh prewarmed broth. These diluted cultures were incubated on an inclined turn-table at a speed of 33 r.p.m., in a 37° incubator. After approximately 2 hrs. these cultures had a titre of 2.0-5.0 x 10^8/ml. All cultures were first measured for their total count with a Petroff-Hauser counting chamber. The number of viable bacteria/ml.

Appendix 6 (Cont'd)

was then measured by plating 0.1 ml samples of serial dilutions of the cultures, made in phosphate buffer, in 3.0 ml. of molten soft-agar, on a nutrient agar plate. The colonies are counted after 18-24 hrs. incubation at 30°, 37° or 42° as required. A knowledge of the total count was useful in planning the dilutions for the viable count and for adjusting donor:recipient ratios in the mating mixtures.

When cultures reached the required total count they were maintained at this figure by packing ice around the bottles or flasks containing the culture. This may be used to maintain donor strains in mid-log phase for up to 2 hrs. with no ill effects on the mating experiment. The iced cultures are allowed to equilibrate to temperature used in the cross for 5 min. before adding the other parent of the cross.

C. Mating procedures

(i) Standard Hfr X F^- cross

Overnight culture of Hfr parent was subcultured in fresh warm broth and grown with aeration to c.2.0-3.0 x 10^8/ml. Saturated overnight of the F^- recipient, c.2.0-4.0 x 10^9/ml., was diluted $^1/_{10}$ in fresh warm broth immediately before the commencement of mating. Take 0.1 ml samples of both parents for dilutions for the viable counts.

Mate 1.0 ml. log Hfr donor with 4 ml. $^{\circ}$/N/10 F^- recipient strain in 250 ml. Erlenmeyer flask with very gentle aeration at 37°C for 60 min. Dilute and plate for recombinants on the appropriate selective media.

Appendix 6 (Cont'd)

(ii) Standard cross for donor strain harbouring an autonomous sex-factor

Overnight cultures of both donor and recipient strains were sub-cultured in fresh warm broth and grown with aeration to c.2.0-3.0 x 10^8/ml. Take 0.1 ml samples for the viable counts of both parents. Mate 1.0 ml. log donor culture with 9.0 ml. log recipient in 250 ml. flask at 37° for 60 min. with very gentle aeration. Dilute and plate for the transfer of the extrachromosomal elements and for chromosomal recombinants.

(iii) Cross-streak mating technique

This can be used to test the fertility of an Hfr donor. Make a broad streak of a log broth culture of a suitable recipient strain on a supplemented minimal agar plate, using either streptomycin or spectinomycin to contraselect the donor strain. Streak log cultures of the donor strains across the recipient culture streak. Incubate the plates for 24 hr. at 37°. This test is easily modified to test for the transfer of F' factors or to check whether a culture contains a derepressed R-factor.

NOTE Nalidixic acid cannot be used for this test, because it inhibits the transfer of both chromosomal and extra-chromosomal material.

(iv) Plate mating technique

The colonies to be tested for their donor ability are patched on a master-plate, 50 patches per plate and incubated overnight at 37°.

Appendix 6 (Cont'd)

The master plates are then replica plated to duplicate plates containing the same medium as the master plate, these replicas are incubated for 3 hrs. at 37°, agar side down, to obtain fresh "log" patches. The duplicate replica plates are then plate-mated to supplemented minimal plates which have been spread with 0.2 ml. of a log broth culture of a suitable $\underline{F}^-$ recipient. The duplicate matings should give identical results, again nalidixic acid cannot be used.

D.(i) The isolation of donor strains

(a) All the fertility derepressed plasmids will successfully infect most recipient strains under the conditions of a standard cross described in Appendix C(ii).

(b) HFCT or HFRT donor cultures. In order to infect strains with fertility repressed plasmids it is necessary to temporarily derepress the fertility system of the plasmids in HFCT or HFRT donor preparations. These are prepared by mixing c.5.0 x 10^4/ml. log phase donor cells with c.1.0 x 10^6/ml. log phase intermediate donor cells, in 5 ml. nutrient broth, and incubating together overnight without aeration at 37°. The overnight mixed culture is diluted $^1/_{20}$ in fresh warm broth and incubated with aeration at 37° for $2\frac{1}{2}$ hrs, the majority of the intermediate donors have been recently infected with the plasmid and will thus serve as highly efficient donors of the sex factor.

Appendix 6 (Cont'd)

D(ii) The recognition of infected recipient bacteria

(a) F^+ bacteria

The presumed infected recipient bacteria can be selected on nutrient or supplemented minimal media using streptomycin, spectinomycin or nalidixic acid to contraselect the original donor strain. After 18 hr. growth at 37°, the colonies are replica plated on to EMB-O plates which have previously been spread with 0.2 ml of M.S.2 (c.2.0 x 10^{12} p.f.u/ml.) and incubated overnight at 42°. Colonies of F^+ bacteria replica plate very poorly or not at all with the precipitation of the EMB dyes in the immediate vicinity of the F^+ colony. F' factors can be tested this way if for any reason they cannot be selected directly, for example the recognition of an $F'lac^+$ in a chromosomally lac^+ background.

(b) Col^+ bacteria

Plate 0.1 ml aliquots of dilutions of the infection mixture in 3.0 ml. of molten soft agar, on oxoid nutrient or supplemented minimal plates. When the first layer has set, a second layer of soft agar is poured over the first to prevent any surface colonies from bursting through the first layer and being washed over the surface of the plates when the layer of soft agar, containing the indicator bacteria, is added.

Incubate the plates overnight at 37°, then overlay with 3.0 ml. soft agar containing 0.1 ml. °/N culture of Col-sensitive indicator bacteria. Incubate the plates at 37° overnight, the Col^+ bacteria are recognised by the zones of inhibition surrounding them.

Appendix 6 (Cont'd)

NOTE It is important to match the antibiotic resistances of the infected recipient and the Col-sensitive indicator strain.

(c) R^+ bacteria

Bacteria infected with R-factors can be selected directly on nutrient or supplemented minimal plates containing the suitable combination of antibiotics.

E(i) The isolation of thymine requiring mutants

A modification of the method of Stacey & Simson (1965) was used to isolate thy^- mutants. The concentrations of trimethoprim and thymine were increased to 200 µg/ml., after 48 hr. incubation, dilutions were plated on nutrient plates supplemented with thymine 50 µg/ml. and trimethoprim 50 µg/ml. Only thymine requiring bacteria can grow on these plates.

E(ii) The isolation of recA mutants

To construct a recA strain, a thyA mutant was first isolated as in Appendix 6 E(i). The thyA strain was crossed with Hfr JC5088, a derivative of Hfr KL16 thr ilv recA56 spcA which transfers thy^+ early. Selection was made for thy^+ recombinants in a 15 minute cross, the purified thy^+ recombinants were then tested for recA.

To test for the recA mutation, streak or replica plate the colonies to be examined onto oxoid nutrient agar plates containing 3 parts per 10,000 of methyl methane sulphonate (MMS), rec^- strains are very sensitive to MMS.

Appendix 7

Procedures used in the experimental sections

A(i) The efficiency of rec⁻ strains as recipients was measured by the standard Hfr cross (see Appendix 6 C(i)). Phenocopies were produced by taking a very heavy innoculum for nutrient agar plates with a fresh overnight growth at 37° and resuspending this heavy growth in nutrient broth breaking up the cell clumps with a Whirlimixer before mating.

(ii) Extrachromosomal transfer by rec⁻ donor strain was measured by the standard cross for autonomous plasmids (see Appendix 6 C(ii)). For the HFCT or HFRT donor preparations, the original F⁻ strain was used as the intermediate donor for example.

AB2463 Col B1⁺ X AB2463 F⁻ X W1655 nalA F⁻

(iii) Chromosomal transfer from rec⁻ donors

As described in Appendix 6 C(ii) and 7 A(ii). The crosses were set up in at least triplicate for all the rec⁻ donors to ensure a reasonably large number of recombinants were examined. Plates were picked at random from each cross and all the recombinants were patched and tested to show that they were genuine recombinants.

(iv) Trypsin treatment

After comparing the number of recombinants produced by Col⁺ donor strains when they were mated with Col-sensitive recipient bacteria in presence or absence of trypsin 200 µg/ml., it was decided not to use trypsin to inactivate free colicin in all subsequent crosses, as the effect of colicin was negligible.

Appendix 7 (Cont'd)

B. The stimulation of chromosome transfer

(i) U.V. irradiation

The output of the U.V. lamp was adjusted to give a dose of 5 ergs/mm^2/sec. The donor bacteria were irradiated in phosphate buffer, in a glass petri dish, the total U.V. dose being 100 ergs/mm^2. After irradiation the donor bacteria were quickly transferred into foil wrapped screw-cap bottles and reconstituted as a nutrient broth. The U.V. dose usually lowered the survival to about 30% except in the case of the Col Ib type factors which confers some protection to their host bacteria. The post-irradiation incubation period was always 45 minutes in nutrient broth at 37°.

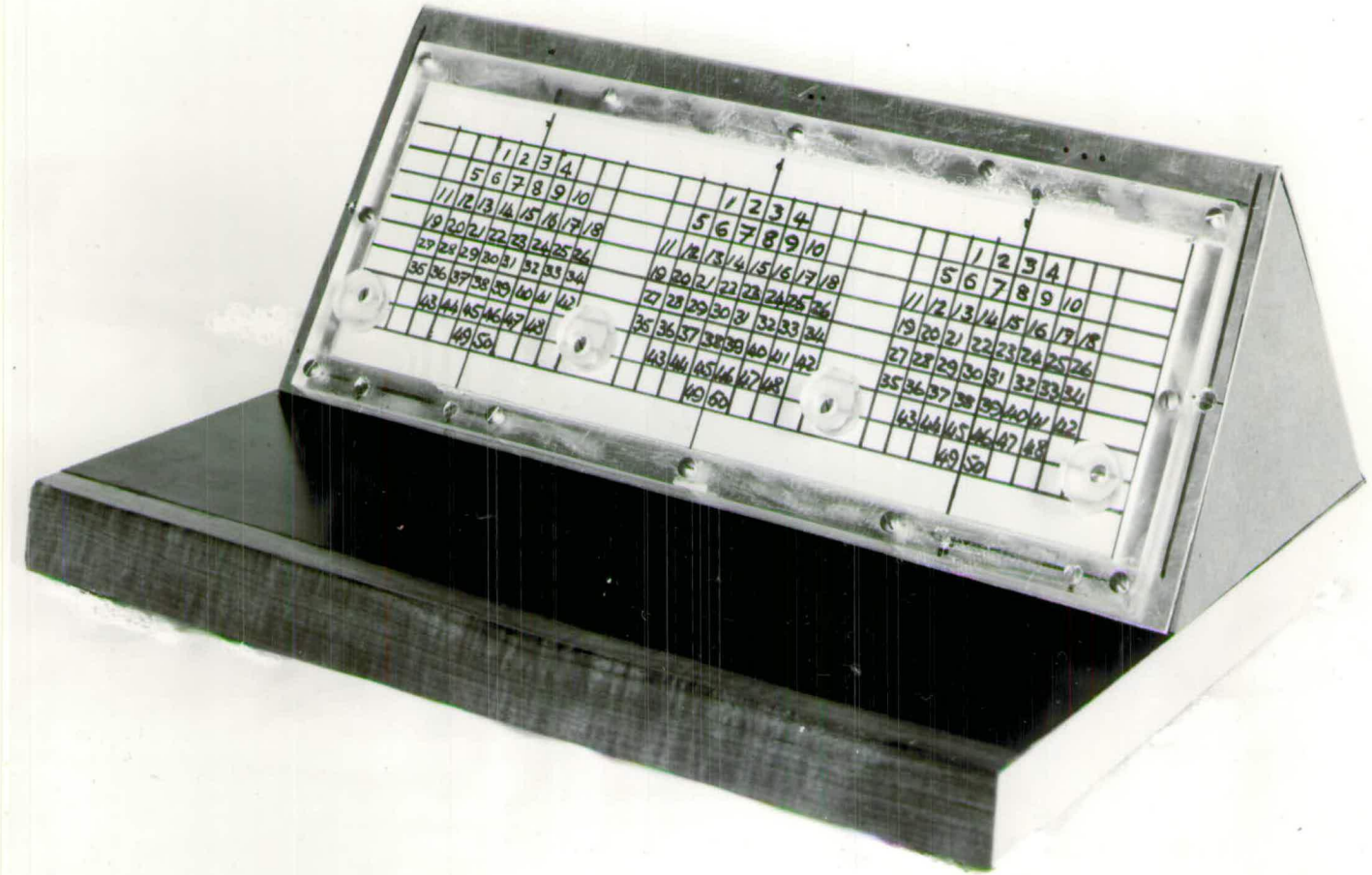
(ii) Newly infected donor bacteria

The strains used are described in Tables 16 and 21. New infection was achieved by mixing c.5.0 x 10^4/ml log phase Primary donor with c.1.0 x 10^6/ml. log phase Secondary donor and incubating the mixture together for 2 hrs. at 37° with gentle aeration. Sub-culture $^1/_{10}$ into fresh warm broth containing spectinomycin 100 µg/ml. and incubate 60 min. at 37° with gentle aeration. This is now a newly infected donor culture, mate with a recipient in a standard 1.0 ml donor X 9.0 ml. F^- recipient ratio.

Appendix 8

A simple device for making multiple master-plates for replica plating, and for reading the results of replica-plating.

See attached photograph.



ACKNOWLEDGEMENTS

I would like to thank Prof. W. Hayes for his advice and encouragement, not only during the last three years and in the preparation of this thesis, but also for the many happy years and opportunities I have had in his MRC Units both in London and in Edinburgh.

I am grateful to Dr. E. Meynell for her generous gift of the fertility derepressed R^+ strains, to Dr. H. Ozeki for the Col Ib^+ drd strain and to Dr. S. Howarth-Thompson for the culture of the Col Ib mutant strain uvp-2. Also my thanks to Drs. N. S. Willetts, P. M. A. Broda and M. Achtman who have always been most helpful in discussions, generous with their unpublished data and patient when I have raided their culture collections.

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ABSTRACT OF THESIS

Name of Candidate Eric Edward Marshall Moody

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Title of Thesis The Mechanism of Chromosome Transfer Mediated by Various Sex Factors in Escherichia coli K12

Many sex-factors or transmissible plasmids are capable of transferring donor chromosomal genes during conjugation with recipient bacteria. This chromosome transfer is believed to result from the interaction of the sex-factor with the bacterial genome to form some type of physical association between the two, linking the genetic material of the bacterium to the transfer mechanism of the sex-factor. The results of crosses performed using recombination-deficient (recA) donor strains suggest that the great part of chromosome transfer by the majority of transmissible plasmids is dependent on the functional integrity of the bacterial recombination (REC) system. Virtually all transfer is abolished when the donor strain is defective in the REC system as the result of a mutation in the recA gene. At the same time there is an indication of an alternative mechanism of transfer which is apparently independent of the REC system.

Most plasmid-chromosome interactions are perfectly adequate for the transfer of donor chromosomal material but rarely result in the reciprocal genetic exchange necessary for the formation of Hfr-type donors.

Chromosome transfer can be stimulated both by U.V. irradiation and by new infection of donor strains, and it is proposed that this stimulation is due to the induction of a plasmid-specific function which promotes interaction with the bacterial chromosome, resulting in the transfer of donor chromosomal genes.

A simple model for chromosome transfer by autonomous plasmids is proposed where the plasmid-specific function would normally interact with the bacterial REC system to join the genomes of the bacterium and the sex-factor in a non-reciprocal recombination event producing a structure capable of single-stranded chromosome transfer.

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