INACTIVATION OF CHROMOSOMAL FRAGMENTS TRANSFERRED FROM Hfr STRAINS*

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AT least three different genes—*recA*, *recB*, and *recC*—are known to determine recombination in *Escherichia coli*. All multiple *rec*$^-$ strains carrying a *recA* mutation are similar to the strain which carries a *recA* mutation alone in regard to their high ultraviolet (UV) sensitivities, high recombination deficiencies, and inabilities to induce phage λ in a lysogen. But these multiple *rec*$^-$ strains show the low level of UV-induced deoxyribonucleic acid (DNA) breakdown which is characteristic of strains carrying a *recB* or *recC* mutation alone. The strain carrying both *recB* and *recC* mutations is similar in all properties to the single mutants. It seems that in a Rec$^+$ strain, the *recA*$^+$ product acts to inhibit DNA breakdown determined by the *recB*$^+$ and *recC*$^+$ products (WILLETTS and CLARK 1969). The strain carrying a *recB* or *recC* mutation was shown to lack a certain exonuclease activity which exists in a Rec$^+$ strain (BUTTIN and WRIGHT 1968; OISHI 1969; BARBOUR and CLARK 1970). Transfer of genetic material has been revealed to occur normally to single or multiple *rec*$^-$ strains as well as to a Rec$^+$ strain by zygotic induction and by formation of F-prime (F′) merodiploids (WILLETTS and CLARK 1969). But the functioning of a newly transferred *lacZ*$^+$ gene in *lacZ* recipients carrying various mutations in the *recA* and *recB* genes was progressively inactivated, unless the *lacZ*$^+$ gene was contained in an episome such as F′ (DUBNAU and MAAS 1969).

The change in activity of transferred fragments of the Hfr chromosome or F′ factors in recipients which are lysogenic for λ*h*$^+$ and carry a mutation or mutations in any one or two of three *rec* genes was examined quantitatively by measuring the number of zygotes which, upon induction, produced phage particles resulting from the λ*h* prophage transferred from the donor.

MATERIALS AND METHODS

Nomenclature: Genetic symbols used to designate genotypes and phenotypes are those of TAYLOR and TROTTER (1967). Resistance and sensitivity to streptomycin and phage T6 are denoted by *r* and *s*, respectively.

Bacterial strains: Bacterial strains were all derivatives of *Escherichia coli* K12. The Hfr strain was W3020 (from Dr. KADA), which transfers its chromosome in the order of *gal–att*λ*–trp*. The F′ strain was W3350 (LEDERBERG 1960) carrying F′ *gal*$_3$ factors, with the chromosomal segments including the *gal* and *att*λ locus (OHKI and TOMIZAWA 1968). These two strains are *str*^{s} *tsx*^{s}, and nonpermissive for growth of λ*sus* mutants (*sup*$^-$). AB1157, AB2463, AB2470 (HOWARD-

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FLANDERS and THERIOT 1966), and JC5474 (WILLETTS and MOUNT 1969) were obtained from Dr. H. OGAWA and were used as a *rec*+, *recA13*, *recB21*, *recC22* recipient, respectively; they are all F⁻ *gal*⁻ *str*^{r} *tsx*^{r} and permissive for growth of λ*sus* mutants (*sup*+). AB1157-A carrying *recA41* (OGAWA, SHIMADA and TOMIZAWA 1968), AB2470-A carrying *recA41* and *recB21*, and JC5474-A carrying *recA41* and *recC22* were made by crossing HfrC53 (from Dr. H. OGAWA) carrying *recA41* with AB1157, AB2470, and JC5474, respectively. AB2470-C carrying *recB21* and *recC22* was made by selecting a *recC22* transductant of AB2470 infected with P1*vir1* (IKEDA and TOMIZAWA 1965) grown on JC5474. They are all F⁻ *gal*⁻ *str*^{r} *tsx*^{r} and *sup*+. As a *rec*+ recipient, a T6-resistant derivative of 594 (WEIGLE 1966), F⁻ *gal*⁻ *str*^{r} *tsx*^{r} and *sup*⁻, was used. After lysogenization with derivatives of λ, all donor and recipient strains were made resistant to λ. The selective indicator for the host-range (*h*) mutant of λ was a T6-resistant derivative of CR63 (APPLEYARD, MCGREGOR and BAIRD 1956), which is resistant to wild-type λ.

Phage strains: Phage strains were all derived from λ. λ*cIts* is λ*cItsI*-1 described by HORIUCHI and INOKUCHI (1967) and it produces a temperature-sensitive immunity repressor. The lysogens are stable at 36°C but can be induced by heating at 47.5°C for 10 min. λ*susA11*, λ*susB1*, λ*susE4*, and λ*susR5* are suppressor-sensitive mutants described by CAMPBELL (1961). λ*h* is a mutant which can infect CR63. Recombinant phages were made by appropriate crosses.

Media: Penassay broth contains 17.5 g of Difco Bacto-Penassay broth in 1 liter of water. λ-broth contains 10.0 g of Polypeptone (Daigo-Eiyo Chemicals, Tokyo) and 2.5 g of NaCl in 1 liter of water. For plating, λ-broth was solidified with 0.6% agar for the top layer and 1% agar for the bottom layer. The EMB medium was supplemented with 1.5% agar and 1% galactose.

Experimental procedures: Exponentially growing cultures of donor and recipient bacteria in Penassay broth were mixed at a titer, based on colony-forming ability, of 2×10^7 to 1×10^8 donors per ml and 4×10^7 to 2×10^8 recipients per ml. Anti-λ serum (K=1) was added to the mating mixture to inactivate free phage particles. After 40 min incubation at 36°C, a portion of the mating mixture was withdrawn, diluted, and agitated vigorously by a Thermo-mixer (Thermonics Co., Tokyo) to interrupt mating.

To measure zygotic induction, the agitated mixture was diluted and plated on λ-agar with melted soft agar containing indicator bacteria and streptomycin. To measure Gal+ recombinants and lysogenic zygotes which produced phage particles of the donor type upon induction, the agitated mixture was diluted 100- to 1000-fold in Penassay broth containing 100 μg per ml of streptomycin and anti-λ serum (K=1), and incubated at 36°C. At intervals thereafter portions of the diluted mixture were withdrawn, diluted, and plated on EMB galactose plates containing streptomycin when Gal+ recombinants were selected. Or they were heated at 47.5°C for 10 min and after dilution plated on λ-agar with melted soft agar containing selective indicator bacteria and streptomycin when the number of zygotes which produced phage particles of the donor type upon induction were examined.

RESULTS

In the experiments to be described below the donor strains used were *sup*⁻: an Hfr strain carrying λ*hcItssusE4* and an F′ strain carrying λ*hcItssusA11* on the episome. The recipient strains were all *sup*+ and lysogenic for λ*cIts*. The λ phage carried by the donors could grow only in zygotes upon induction. The indicator strain was a derivative of CR63 which is *sup*+ and resistant to wild-type λ but sensitive to λ*h*. Only the λ phage transferred from the donor could grow on the indicator strain. By measuring the number of zygotes which produced phage particles of the donor type (*h*) upon induction, the activity of prophage in zygotes transferred from the donor could be examined.

The donor strains were mated with various recipient strains for 40 min. The number of zygotes which produced phage particles of the donor type upon in-

TABLE 1

Recipient ability of various rec⁻ *strains*

Recipient strain	Genotype *recA*	*recB*	*recC*	Donor strain: Hfr A	Hfr B	F′ A	F′ B	F′ C
AB1157	+	+	+	1	1	1	1	1
AB1157-A	41	+	+	1/4	1/20	1	1/20	1/2
AB2470	+	21	+	.	2/3	.	1	.
JC5474	+	+	22	.	1/5	.	1/5	.
AB2470-A	41	21	+	.	1/5	.	1/4	.
JC5474-A	41	+	22	.	1/8	.	1/5	.
AB2470-C	+	21	22	.	2/3	.	1	.

The values shown are ratios of the frequency per donor obtained with each *rec*⁻ recipient in 40 min mating to that with a Rec+ recipient.

(A) The ability of a *recA* recipient to produce phage particles by zygotic induction in a cross with W3020(λ) or W3350 F′(λ).

(B) The ability of various *rec*⁻ recipients carrying λ*cIts* to form zygotes which produced phage particles of the donor type upon induction in crosses with W3020(λ*hcItssusE4*) or W3350 F′(λ*hcItssusA11*).

(C) The ability of a *recA* recipient carrying λ*cIts* to form gal+/gal⁻ merodiploids in a cross with W3350 F′(λ*hcItssusA11*).

duction was then measured, and the frequency per donor for each cross was calculated. The values in Table 1-B are shown as the ratio of the frequency per donor obtained in the mating of the donor with each *rec*⁻ recipient to that with a Rec+ recipient. The abilities of the *recB* and *recBrecC* strains were almost as great as that of the Rec+ strain, while the abilities of the other strains were lower. The strain with the lowest ability was the *recArecB*+*recC*+ strain. Transfer of λ prophage seems to have occurred normally to these *rec*⁻ strains, because zygotic induction and F′ merodiploid formation have been shown to occur at similar frequencies in Rec+ and various *rec*⁻ strains. Though transfer of λ prophage to the *recA* recipient seems to have occurred almost normally (Table 1-A and C), the ability of the *recA* recipient to form zygotes which could produce phage particles of the donor type was very low. Similar results were obtained with other *recA* recipients carrying a *recA1* (CLARK and MARGULIES 1965), *recA13* or *recA42* (OGAWA, SHIMADA and TOMIZAWA 1968) mutation. The observed low ability is partly due to low efficiency of induction with heat or rapid inactivation of transferred fragments before and during heat treatment.

The change with time after interruption of 40 min mating in number of zygotes which produced phage particles of the donor type upon induction was then examined. The results obtained from crosses of an Hfr strain with various recipient strains are shown in Figure 1. The number of zygotes which produced phage particles of the donor type upon induction decreased gradually during the postconjugation period at similar rates in all *rec*⁻ recipients except a *recA* recipient, and in 120 min it reached a level 10% to 40% of that at interruption of mating. It decreased very rapidly in the case of a *recA* recipient. Similar results were obtained from experiments in which other *recA* recipients carrying a *recA1*, *recA13*, or *recA42* mutation were used. The number was almost constant at least

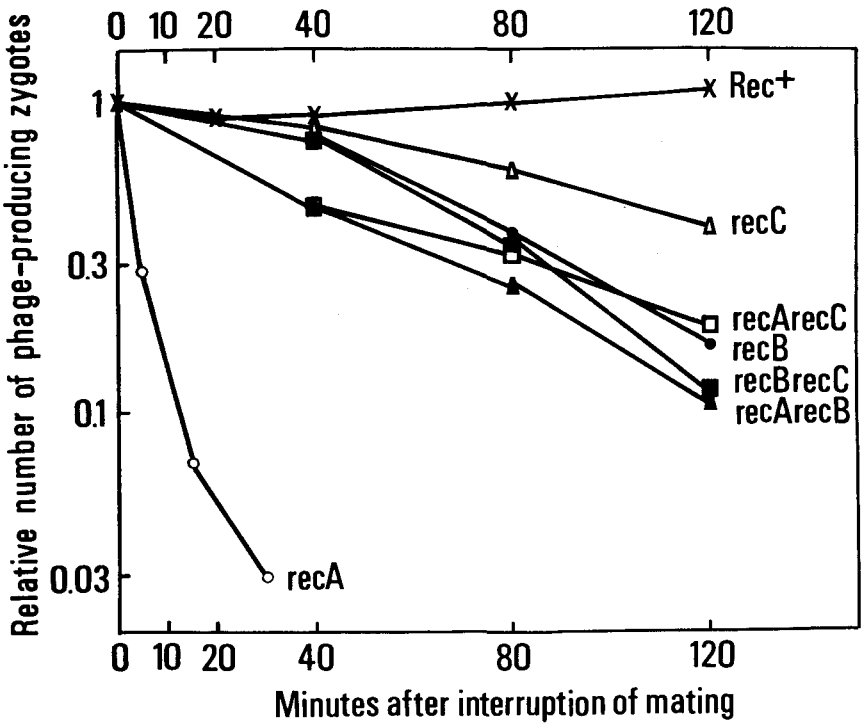

FIGURE 1.—Change in relative number of zygotes which, upon induction, produced phage particles derived from the λ prophage carried by the donor as a function of the time after interruption of 40 min mating with an Hfr strain.

Strains used were as described in Table 1(B). After vigorous agitation, the mating mixture was diluted 100- to 1000-fold in Penassay broth containing streptomycin and incubated at 36°C. At intervals, portions of the diluted mixture were withdrawn, heat-induced, and plated. ○ *recA41*; ● *recB21*; △ *recC22*; ▲ *recA41recB21*; □ *recA41recC22*; ■ *recB21recC22*; × Rec+.

during 120 min postconjugation period in a Rec+ recipient. The total number of recipient cells increased from the start of mating, with a lag at the beginning, in all the recipient strains. The initial slow rate of growth of recipient cells was also observed when unmated recipient cells were treated in the same way. When an F′ strain was used as a donor, the number of zygotes which produced phage particles of the donor type upon induction increased with time in all crosses, including Rec+ and various *rec*⁻ recipients, as the total number of recipient cells increased (Figure 2). Transferred F′ factors seem to have been protected against inactivation and to have multiplied autonomously. We conclude from the above results that transferred fragments of the Hfr chromosome were inactivated with time in zygotes, very rapidly in a *recA* recipient and gradually in other *rec*⁻ recipients.

The inactivation of transferred fragments of the Hfr chromosome in a *recA* recipient could occur either before and after or only after interruption of mating. If transfer of the Hfr chromosome to a *recA* recipient occurred at the rate meas-

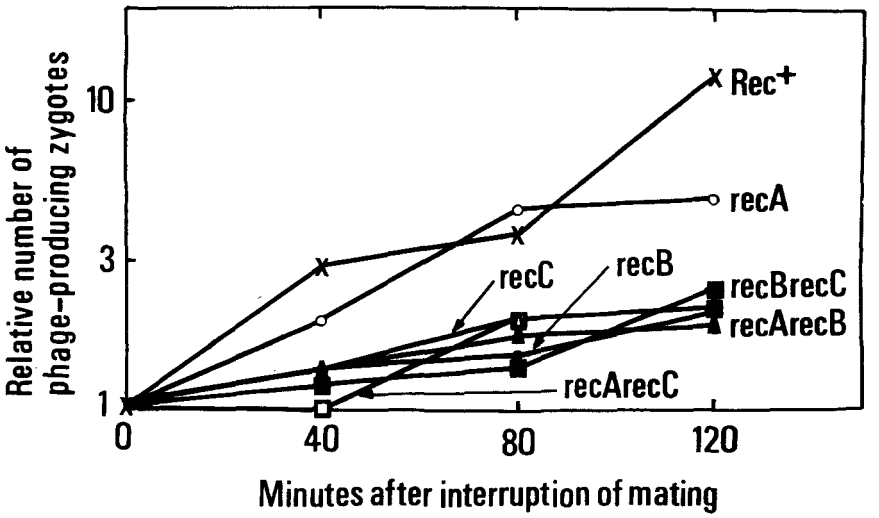

FIGURE 2.—Change in relative number of zygotes which, upon induction, produced phage particles derived from the λ prophage carried by the donor as a function of time after interruption of 40 min mating with an F′ strain.

Strains used are described in Table 1(B). Experimental procedures were as described in Figure 1.

ured by zygotic induction and if inactivation occurred simultaneously with transfer at the rate measured after interruption of mating, the number of zygotes which produced phage particles of the donor type upon induction is expected to increase soon after transfer of prophage begins, since more prophage are expected to be transferred than inactivated per unit time. Later, as the number of prophage transferred per unit time decreases, the number of phage-producing zygotes is expected to reach a maximum. The rate of decrease of the number of phage-producing zygotes is then expected to approach that of prophage inactivation.

The kinetics of transfer of prophage was examined by zygotic induction in an interrupted mating of a lysogenic Hfr donor with a nonlysogenic *recA* recipient (the open triangles in Figure 3). The rate of transfer of prophage was about 5×10^5 phage-producing zygotes per ml formed per minute and transfer stopped about 100 min after the beginning of mating. The change in number of zygotes which produced phage particles of the donor type upon induction was simultaneously examined in an interrupted mating of the same lysogenic Hfr donor and a lysogenic *recA* recipient (the open circles in Figure 3). The number of phage-producing zygotes in 10 min incubation after interruption of mating was also examined (the filled circles in Figure 3). The fraction of the transferred prophage which escaped from inactivation in 10 min incubation after interruption of mating was about 1/5.

From the rate of transfer and the rate of inactivation of prophage, the expected maximum number of zygotes which produced phage particles of the donor type can be approximately calculated on the above assumptions and is 3×10^6 per ml.

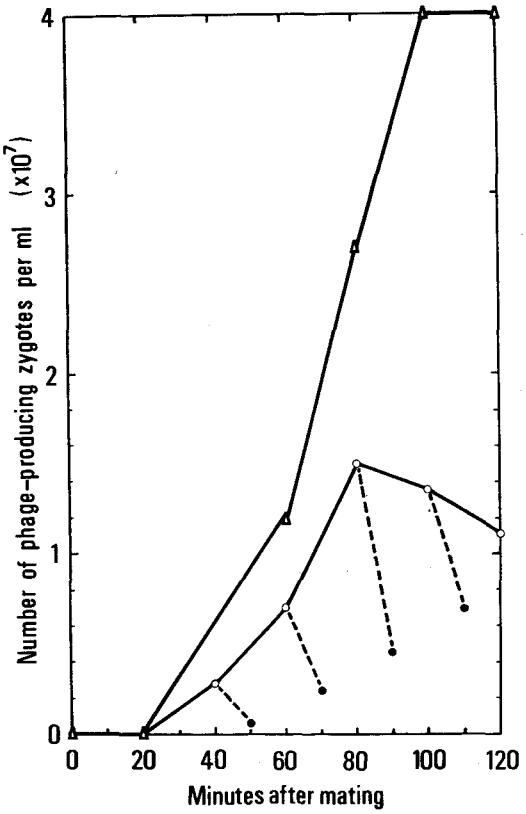

FIGURE 3.—Change in number of zygotes which produced phage particles of the donor type upon induction in a prolonged mating of W3020(λ*hcItssusA11*) with AB2463(λ*cIts*).

At a given time, a portion of the mating mixture was withdrawn and agitated vigorously. Heat-induction and plating were performed before (○) and after (●) 10 min incubation at 36°C. λ zygotic induction (△) in a cross of W3020(λ) with AB2463 resistant to λ is also shown.

Although the experimental result shows a maximum, the observed value (1.5×10^7 per ml) is much higher than the expected one. Transfer of prophage from the Hfr donor stopped about 100 min after the beginning of mating. If inactivation of prophage occurred simultaneously with transfer, the rate of decrease of the number of phage-producing zygotes without interruption of mating should be similar to that after interruption of mating. But the observed decrease without interruption of mating from the 100 min point to the 120 min point in Figure 3 is much smaller than that after interruption of mating. It can be concluded that the inactivation before interruption of mating did not occur at as high a rate as that after interruption of mating. It suggests that the inactivation occurred only after interruption of mating. Observed lower number of phage-producing zygotes in the cross with the lysogenic *recA* recipient as compared with the number of phage-producing zygotes as measured by zygotic induction may be due to spontaneous interruption of mating.

The inactivation of chromosomal fragments transferred from an Hfr donor seems not to have occurred in a Rec$^+$ recipient (Figure 1). But a possibility exists that the increase in number of infective centers caused by recombination, that is, integration into and increase with the recipient chromosome of the λ prophage on a transferred fragment of the Hfr chromosome, may have compensated for the decrease in number of infective centers due to inactivation of λ prophage on a transferred fragment of the Hfr chromosome. To examine the putative decrease in number of zygotes which could produce phage particles of the donor type upon induction in a Rec$^+$ recipient, the following experiments were performed.

In the experiment to be described below, the Hfr strain used was *sup*$^-$ and lysogenic for *λhcItssusA11*. The Rec$^+$ recipient strain used was also *sup*$^-$ and lysogenic for *λcItssusB1susR5*. The selective indicator was CR63. Differing from the previous experiments, zygotes can produce phage particles upon induction only when they carry both the *susA* and the *susBsusR* mutants or the wild-type Sus$^+$ recombinants. But formation of the wild-type recombinant on the recipient chromosome is expected to be strongly suppressed, as these three *sus* mutations are situated close to each other and in the order *susR–susA–susB* on the prophage map of λ.

The result of a cross of an Hfr strain with a Rec$^+$ strain is shown in Figure 4. The total number of recipient cells increased from the start of mating with a slight lag. The number of zygotes which produced phage particles upon induction decreased gradually after interruption of 40 min mating and reached in a period of about four generations its minimum value of 30% of the original value at interruption of mating. Thereafter it increased, with a slight lag, at the same rate as the number of total recipient cells. The change in number of Gal$^+$ recombinants in the same cross is also shown in Figure 4, and it is essentially similar to that of Lac$^+$ recombinants (TOMIZAWA 1960) and Gal$^+$ recombinants (JACOB and WOLLMAN 1961). As only the number of stable Gal$^+$ recombinants was measured, it is quite natural that there is no decrease in the number of Gal$^+$ recombinants. Essentially similar results were obtained from experiments of the same type in which phage T6 was added to the mating mixture at a high multiplicity of infection to eliminate donor cells completely at the time of interruption of mating. We conclude that transferred fragments of the Hfr chromosome in zygotes were gradually inactivated with time even in a Rec$^+$ recipient.

DISCUSSION

It was shown that the number of zygotes which produced phage particles resulting from the λ prophage on transferred fragments of the Hfr chromosome upon induction gradually decreased with time after interruption of mating. When an F′ strain was used as a donor, no such decrease was observed. The result was similar to those reported by DUBNAU and MAAS (1969) who studied the expression of newly transferred *lacZ*$^+$ genes in *lacZ* recipients carrying various mutations in the *recA* and *recB* genes.

We interpret our results as follows. The Hfr DNA was transferred as a single

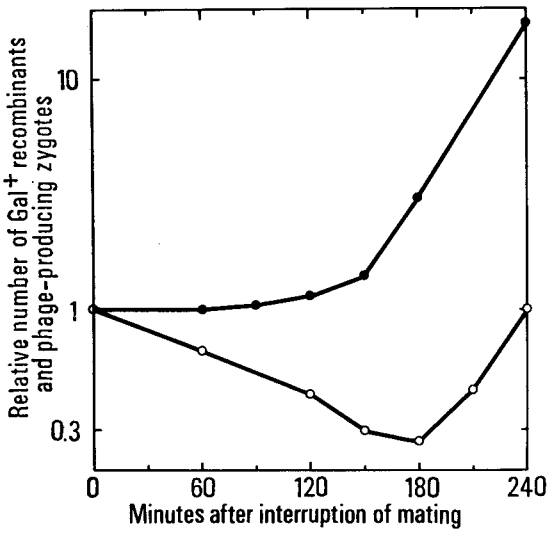

FIGURE 4.—Change in relative number of Gal+ recombinants and zygotes which produced phage particles of the donor type upon induction as a function of time after interruption of 40 min mating with an Hfr strain.

The donor strains used were W3020(λ*hcItssusA11*). The recipient strain used was 594 (λ*c Its susB1susR5*) *tsx*^{r}. Experimental procedures were described in Figure 1. Gal+ recombinants were also selected on EMB agar plates supplemented with galactose and streptomycin.

○ Zygotes which produced phage particles of the donor type upon induction; ● Gal+ recombinants.

strand and its complementary strand was synthesized rapidly in the recipient (OHKI and TOMIZAWA 1968). The resulting double-stranded fragments of the Hfr chromosome were inactivated with time in zygotes. The rate of inactivation is very high in a *recA* recipient. This rapid inactivation caused by a *recA* mutation was mostly prevented by a *recB* or *recC* mutation. This relation between a *recA* mutation and a *recB* or *recC* mutation is quite similar to that with regard to UV-induced or spontaneous DNA degradation (WILLETTS and CLARK 1969). It seems, therefore, that the wild-type *recB* and *recC* products give rise to rapid inactivation of transferred fragments of the Hfr chromosome in zygotes, and that in a Rec+ recipient, the wild-type *recA* product prevents this rapid inactivation. Though the observed inactivation could be due either to physical destruction of transferred fragments or to inhibition of their functional activity, the above interpretation suggests that the inactivation may be due to physical destruction, at least in a *recA* recipient. The inactivation in a *recA* recipient seems to occur only after interruption of mating. The wild-type *recB* and *recC* products may rapidly inactivate transferred fragments of the Hfr chromosome acting at their distal ends created by mechanical breakage, and the wild-type *recA* product may protect the distal ends from this action of the wild-type *recB* and *recC* products.

The nature of inactivation in a Rec+ strain and *rec*$^{-}$ strains other than a *recA* strain is obscure. The inactivation may have been due to a cause not related to *rec* genes.

The result obtained with a Rec$^+$ recipient showed that transferred fragments of the Hfr chromosome in Rec$^+$ zygotes were gradually inactivated with time after interruption of mating. This does not conflict with the results reported by LEDERBERG (1957) and TOMIZAWA (1960) who showed that segregations of genetically pure recombinants occurred chiefly within four generations after the entrance of the genetic markers scored, and that repeated recombinations were rare events. It may be quite natural that no report of abortive conjugation even in a Rec$^+$ strain has ever appeared in the literature, as inactivation of transferred fragments of the Hfr chromosome in zygotes seems to be a good enough reason for the absence of abortive conjugation.

Transferred fragments of the F′ factor may be protected against inactivation by forming a circular structure, while transferred fragments of the Hfr chromosome are linear. But not all transferred fragments of the F′ factor seem to be stable in these *rec*$^-$ recipients, as the frequencies of F′ merodiploid formation measured by selecting Gal$^+$ offspring (data not shown) were lower than the frequencies of transfer of F′ factors as measured by zygotic induction.

There still remain to be considered some possible explanations for the observed decrease in number of zygotes which produced phage particles of the donor type upon induction. The decrease may have been due to (1) loss of ability of zygotes to produce phage particles, or (2) loss of ability of zygotes to synthesize protein in general, or (3) death of zygotes, or spontaneous lethal sectoring (HAEFNER 1968) of whole recipient cells including zygotes. So far these explanations cannot be ruled out except in the case of a *recA* recipient.

An experiment of the same type as shown in Figure 1 was performed, in which the Hfr donor and *recA* recipient cells were mixed in a ratio of one to one. Since a high proportion of recipient cells became zygotes under this condition, one might expect that the ability to produce phage particles upon induction (expected from explanations 1, 2 and 3) or the number and growth rate (expected from explanations 2 and 3) of whole recipient cells in the mating mixture, as compared with those of unmated recipient cells, would be greatly affected and reduced in the case of a *recA* recipient in which the decrease was very rapid. But the observation was not as such (data not shown). Furthermore the decrease in a *recA* recipient was too rapid to be accounted for by spontaneous lethal sectoring, since the frequency of spontaneous lethal sector formation roughly predicted from the rate of decrease of the phage-producing zygotes was too high for bacterial cells to survive and grow.

SUMMARY

The change in activity of transferred fragments of the Hfr chromosome or F′ factors in recipients which are lysogenic for λh^+ and carry a mutation or mutations in any one or two of three *rec* genes—*recA*, *recB*, and *recC*—was examined by measuring the number of zygotes which, upon induction, produced phage particles resulting from the prophage (λh) transferred from the donor. When the donor was an Hfr strain, the number decreased with time very rapidly in a *recA* recipient, and decreased gradually in *recB*, *recC*, *recBrecC*, *recArecB*, and

recArecC recipients. Gradual decrease was observed even in a Rec$^+$ strain. But no decrease was observed when the donor was an F′ strain. We conclude that the functioning of transferred fragments of the Hfr chromosome is progressively inactivated in zygotes, very rapidly in a *recA* recipient, and gradually in other recipients. In a Rec$^+$ recipient, the *recA*$^+$ product seems to inhibit inactivation of transferred fragments of the Hfr chromosome determined by the *recB*$^+$ and *recC*$^+$ products. Gradual inactivation of transferred fragments of the Hfr chromosome in Rec$^+$ zygotes seems to be a good enough reason for the absence of abortive conjugation.

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