INACTIVATION OF CHROMOSOMAL FRAGMENTS TRANSFERRED FROM Hfr STRAINS

by

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CONTENTS

INTRODUCTION

The male phenotype, or donor ability, of cells of Escherichia coli K12 is determined by the presence of an episome called an F factor. Female or recipient cells (F^-) lack this factor. In F^+ donor strains the F factor exists as an autonomously replicating element and is transmissible independently of the host chromosome. In Hfr (yielding high frequency of recombination) donor strains the F factor is integrated into and replicates with the bacterial chromosome. Hfr cells transfer their chromosomes to suitable recipients and chromosomal markers are transferred in a linear, oriented sequence characteristic of the particular Hfr strain, beginning at the site of the F factor integration. The F' factor is an F factor incorporating a fragment of the bacterial chromosome. F' factors behave like F factors; they can exist as autonomous episomes, being transmitted independently of the host chromosome. If in the course of transfer the chromosome breaks, the recipient receives only a fragment of the donor chromosome (Jacob and Wollman 1961; Clark and Adelberg 1962). It seems to be sure that the donor chromosome is transferred as a single strand and acts as a template for synthesis of its complementary strand in the recipient (Cohen, Fisher, Curtiss, and Adler 1968; Ohki and Tomizawa 1968; Rupp and Ihler 1968).

After the transfer of genetic material, genetic recombination between the transferred fragment of the donor chromosome and the recipient chromosome takes place in zygotes. At least three different genes----- recA, recB, and recC-----are known to determine the genetic recombination events. 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 lambda (λ) in a lysogen. But these multiple rec⁻ strains show the low level of UV-induced or spontaneous 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 which is determined by the recB and recC products (Willetts and Clark 1969). The strains carrying a recB or recC mutation were shown to lack certain adenosine triphosphate (ATP)-dependent deoxyribonuclease(DNase) activities which exist in Rec⁺ strains (Buttin and Wright 1968; Oishi 1969; Barbor and Clark 1970; Goldmark and Linn 1970). Transfer of genetic material has been shown to occur normally to single or multiple rec⁻ strains as well as to Rec⁺ strains by zygotic induction and by formation of 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 λh⁺ 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 λh prophage transferred from the donor.

MATERIALS AND METHODS

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

Bacterial strains: Bacterial strains used were all derivatives of Escherichi coli K12. The Hfr strain was W3020 (from Dr. Kada), which transfers its chromosom in the order of gal-attλ-trp. The F' strain was W3350 (Lederberg 1960) carrying F' gal$_3$ factors, with the chromosomal segments including the gal and attλ locus (Ohki and Tomizawa 1968). These two strains are str^s tsx^s, and nonpermissive for growth of λsus mutants (sup$^-$). AB1157, AB2463, AB2470 (Howard-Flanders and Therio 1966), and JC5474 (Willetts and Mount 1969) were obtained from Dr. H. Ogawa and w used as a rec$^+$, recA13, recB21 and recC22 recipient, respectively; they are all F gal$^-$ str^r tsx^r and permissive for growth of some λsus mutants (sup$^+$). AB1157-A car rying recA41 (Ogawa, Shimada and Tomizawa 1968), AB2470-A carrying recA41 and rec B21, and JC5474-A carrying recA41 and recC22 were made by crossing HfrC53 (from D H. Ogawa) carring recA41 with AB1157, AB2470, and JC5474,respectively. To verify the presence of each of the rec$^-$ mutations in these three strains, advantage was taken of the cotransduction of recA with cysC, and of recC and recB with thyA and lysA. P1vir1 (Ikeda and Tomizawa 1965) was grown on each of these rec$^-$ strains an used to transduce AB1157thyA and AT713 (cysC lysA) which was obtained from Dr. H. Ogawa. Among the transductants prototrophic for these markers, Rec$^-$ strains were recognized by their UV sensitivity. AB2470-C carrying recB21 and recC22 was made by selecting a recC22 transductant of AB2470 infected with P1vir1 grown on JC5474 To verify the presence of recB21 and recC22 mutations, the F_{17} factor (Itoh 1968) was used, which covers the recB-recC region. A strain which is made UV resistant by F_{17} recB$^+$recC$^+$ factors, but not by either F_{17} recB$^+$recC22 or F_{17} recB21recC$^+$ factors should carry both recB21 and recC22. AB1157-A, AB2470-A, JC5474-A, and

AB2470-C are all F'

AB2470-C 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. λcIts is λcItsI-1 described by Horiuch 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. λsusA11, λsusB1, λsusE4, and λsusR5 are suppressor-sensitive mutants described by Campbell (1961). λh is a mutant which can infect CR63. Recombinant phages were made by appropriate crosses. The presence of each of the sus$^-$ mutations in recombinant phages was verified by complementation test.

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 X 10^7 to 1 X 10^8 donors and 4 X 10^7 to 2 X 10^8 recipients per ml. Anti-λ serum was added (K=1) 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 agar 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

I. Recipient ability of various rec⁻ strains.

In the experiments to be described in this section and the following sections the donor strains used were sup⁻: an Hfr strain carrying λhcItssusE4 and an F' strain carrying λhcItssusA11 on the episome. The recipient strains were all sup⁺ and lysogenic for λcIts. 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 λh. 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 induction 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 recA1 (Clark and Margulies 1965), recA13 or recA42 (Ogawa, Shimada and Tomizawa 1968) mutation. The observed low ability is partially due to low efficiency of induction with heat or rapid inactivation of transferred fragments before and during heat treatment.

II. Inactivation of transferred fragments of the Hfr chromosome in various rec⁻ recipients.

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 the 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 the 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 the recA recipient. Similar results were obtained from experiments in which other recA recipients carrying recA1, recA13, or recA42 mutation were used. The number was almost constant at least during 120 min postconjugation period in the 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 the 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 coclude 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.

III. Inactivation in recA recipient.

The inactivation of transferred fragments of the Hfr chromosome in the 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 measured by zygotic induction and if inactivation occurred simultaneously with trans-

fer 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 min 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 inetrruption 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 upon induction can be approximately calculated on the above assumptions and is about 3×10^6 per ml. 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 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. 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, followed by inactivation of prophage.

IV. Inactivation in Rec$^+$ recipient.

The inactivation of chromosomal fragments transferred from the Hfr donor seems not to have occurred in the Rec$^+$ recipient (Figure 1). But a possibility exist 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 the λ prophage on a transferred fragment of the Hfr chromosome. To examine the putative decrease in number of zygotes which produced 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 strain was CR63. Differing from the experiments in the preceding sections, zygotes can produce phage particles upon induction only when they carry both the susA and 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 susR5-susA11-susB1 on the prophage map of λ.

The result of a cross of the Hfr strain with the 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 of the donor type upon induction decreased gradually after interruption of 40 min mating and reached

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 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 strand and its complementary strand was synthesized rapidly in the recipient (Cohen, Fisher, Curtiss, and Adler 1968; Ohki and Tomizawa 1968; Rupp and Ihler 1968). The resulting double-stranded fragments of the Hfr chromosome participated in genetic recombination in zygotes carrying recA$^+$ genes, and they were inactivated with time in zygotes irrespective of rec genotypes. 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 those with regard to UV-induced or spontaneous DNA breakdown (Willetts and Clark 1969), and to the ability to support growth of λfec$^-$ (Signer 1971; Zissler, Signer, and Schaefer 1971). 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 mechanism of functioning of the recA product whose absence leads to several pleiotropic effects is as yet unknown.

The nature of inactivation in a Rec^+ strain and rec^- strains other than a recA strain is obscure. The inactivation in a Rec^+ strain may have been residual one by the wild-type recB and recC products escaped from the inhibitory action of the wild-type recA product. The inactivation in a Rec^+ strain and rec^- strains other than recA strain may have been the consequence of some erroneous recombination events. Or the inactivation may have been due to a cause not related to rec genes, such as general exclusion of exogenous DNA by a certain deoxyribonucleolytic function, since there exist several endonucleases and exonucleases in E. coli cells, though their in vivo functions are as yet unknown.

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 the repeated recombinations were rare events. It may be quite natural that no report of 'abortive conjugation'(Dubnau and Maas

1969), unilinear inheritance of transferred fragments of the Hfr chromosome in zygotes, 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 F' factors may be protected against inactivation by forming a circular structure, while transferred fragments of the Hfr chromosome are linear. Or F' factors may be situated in special sites in the bacterial cell, which are inaccessible to inactivation. But not all transferred F' factors 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 measured by zygotic induction.

In contrast with the negative results by Hertman and Luria (1967) and Dubnau and Maas (1969), abortive transduction by phage P1 has been revealed in our laboratory to occur in recA and recB strains as well as in Rec$^+$ strains (Tomizawa and Ogawa 1968; Yamamoto and Tomizawa, unpublished results). The discrepancy may be due to the bacterial strains, genetical markers and experimental techniques used. We believe that chromosomal fragments introduced by phage P1 function properly in recA and recB recipients, even when they are not integrated into the host chromosomes. Again we do not know why they are protected against inactivation.

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 (4) spontaneous lethal sectoring (Haefner 1968) of whole re-

cipient cells including zygotes of various rec⁻ strains. So far these explanations cannot be completely 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 the 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 the recA recipient in which the decrease was very rapid. But the observation was not as such (data not shown). Furthermore the decrease in the 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 a Rec^+ zygotes seems to be a good enough reason for the absence of 'abortive conjugation.'

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LITERATURE CITED

Appleyard, R. K., J. F. McGregor and K. M. Baird, 1956. Virology 2: 565.

Barbour, S. D. and A. J. Clark, 1970. Proc. Natl. Acad. Sci. U.S. 65:955.

Buttin, G. and M. R. Wright, 1968. Cold Spring Harbor Symp. Quant. Biol. 33:259.

Campbell, A. L., 1961. Virology 14:85.

Clark, A. J. and A. D. Margulies, 1965. Proc. Natl. Acad. Sci. U.S. 53:451.

Clark, A. J. and E. A. Adelberg, 1962. Ann. Rev. Microbiol. 16:289.

Dubnau, E. and W. K. Maas, 1969. Mol. Gen. Genet. 103:305.

Goldmark, P. J. and S. Linn, 1970. Proc. Natl. Acad. Sci. U.S. 67:434.

Haefner, K., 1968. J. Bacteriol. 96:652.

Hertman, I. and S. E. Luria, 1967. J. Molec. Biol. 23:117.

Horiuch, T. and H. Inokuchi, 1967. J. Molec. Biol. 23:217.

Howard-Flanders, P. and L. Theriot, 1966. Genetics 53:1137.

Ikeda, H. and J. Tomizawa, 1965. J. Molec. Biol. 14:89

Itoh, T., 1968. Annual Report of Biological Works, Faculty of Science, Osaka University 16:147.

Jacob, F. and E. L. Wollman, 1961. in Sexuality and Genetics of Bacteria. Academic Press, New York.

Lederberg, E. M., 1960. Symp. Soc. Gen. Microbiol. 10:115.

Lederberg, J., 1957. Proc. Natl. Acad. Sci. U.S. 43:1060.

Ogawa, H., K. Shimada and J. Tomizawa, 1968. Mol. Gen. Genet. 101:227.

Ohki, M. and J. Tomizawa, 1968. Cold Spring Harbor Symp. Quant. Biol. 33:651.

Oishi, M., 1969. Proc. Natl. Acad. Sci. U.S. 64:1292.

Signer, E., 1971. in The Bacteriophage Lambda. Cold Spring Harbor Laboratory, New York.

Taylor, A. L. and C. D. Trotter, 1967. Bacteriol. Rev. 31:332.

Tomizawa, J., 1960. Proc. Natl. Acad. Sci. U.S. 46:91.

Tomizawa, J. and H. Ogawa, 1968. Cold Spring Harbor Symp. Quant. Biol. 33:243.

Weigle, J., 1966. Proc. Natl. Acad. Sci. U.S. 55:1462.

Willetts, N. S. and A. J. Clark, 1969. J. Bacteriol. 100:231.

Willetts, N. S. and D. W. Mount, 1969. J. Bacteriol. 100:923.

Zissler, J., E. Signer and F. Schaefer, 1971. in The Bacteriophage Lambda. Cold Spring Harbor Laboratory, New York.

Cohen, A., W. D. Fisher, R. Curtiss, and H. I. Adler, 1968. Cold Spring Harbor Symp. Quant. Biol. 33:635.

Rupp, W. D. and G. Ihler, 1968. Cold Spring Harbor Symp. Quant. Biol. 33:647.

Table 1. Recipient ability of various *rec*⁻ strains

Recipient strain	Genotype			Donor strain				
				Hfr		F'		
	recA	*recB*	*recC*	A	B	A	B	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 are shown as the ratio of the frequency per donor obtained with each *rec*⁻ recipient in 40 minutes' 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*cItssusE4*) 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*cItssusA11*).

FIGURE LEGEND

Figure 1----Change in relative number of zygotes which produced, upon induction, phage particles derived from the λh prophage carried by the donor as a function of time after interruption of 40 minutes' mating with an Hfr strain.

Strains used were as described in Table 1(B). After vigorous agitation, the mating mixture was diluted one hundred- to one thousand-fold in Penassay broth containing streptomycin and incubated at 36°C. At intervals portions of the diluted mixture were withdrawn, heat-induced and plated. o recA41; • recB21; Δ recC22; ▲ recA41recB21; □ recA41recC22; ■ recB21recC22; x Rec^+.

Figure 2----Change in relative number of zygotes which produced, upon induction, phage particles derived from the λh prophage carried by the donor as a function of time after interruption of 40 minutes' mating with an F' strain.

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

Figure 3----Change in number of zygotes which produced phage particles of the donor type upon induction in a prolonged mating of W3020(λhcItssusA11) with AB2463(λcIts).

At a given time, a portion of the mating mixture was withdrawn, agitated vigorously; heat-induction and plating were performed before (o) and after (•) 10 minutes' incubation at 36°C. λ zygotic induction (Δ) in a cross of W3020(λ) with AB2463 resistant to λ is also shown.

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 minutes' mating with an Hfr strain.

The donor strain used was W3020(λhcItssusA11). The recipient strain used was 594(λcItssusB1susR5) tsx^r. Experimental procedures were as described in Figure 1. Gal$^+$ recombinants were also selected on EMB agar plates supplemented with galactose and streptomycin. o zygotes which produced phage particles of the donor type upon induction; • Gal$^+$ recombinants.

Fig. 1

Figure 1

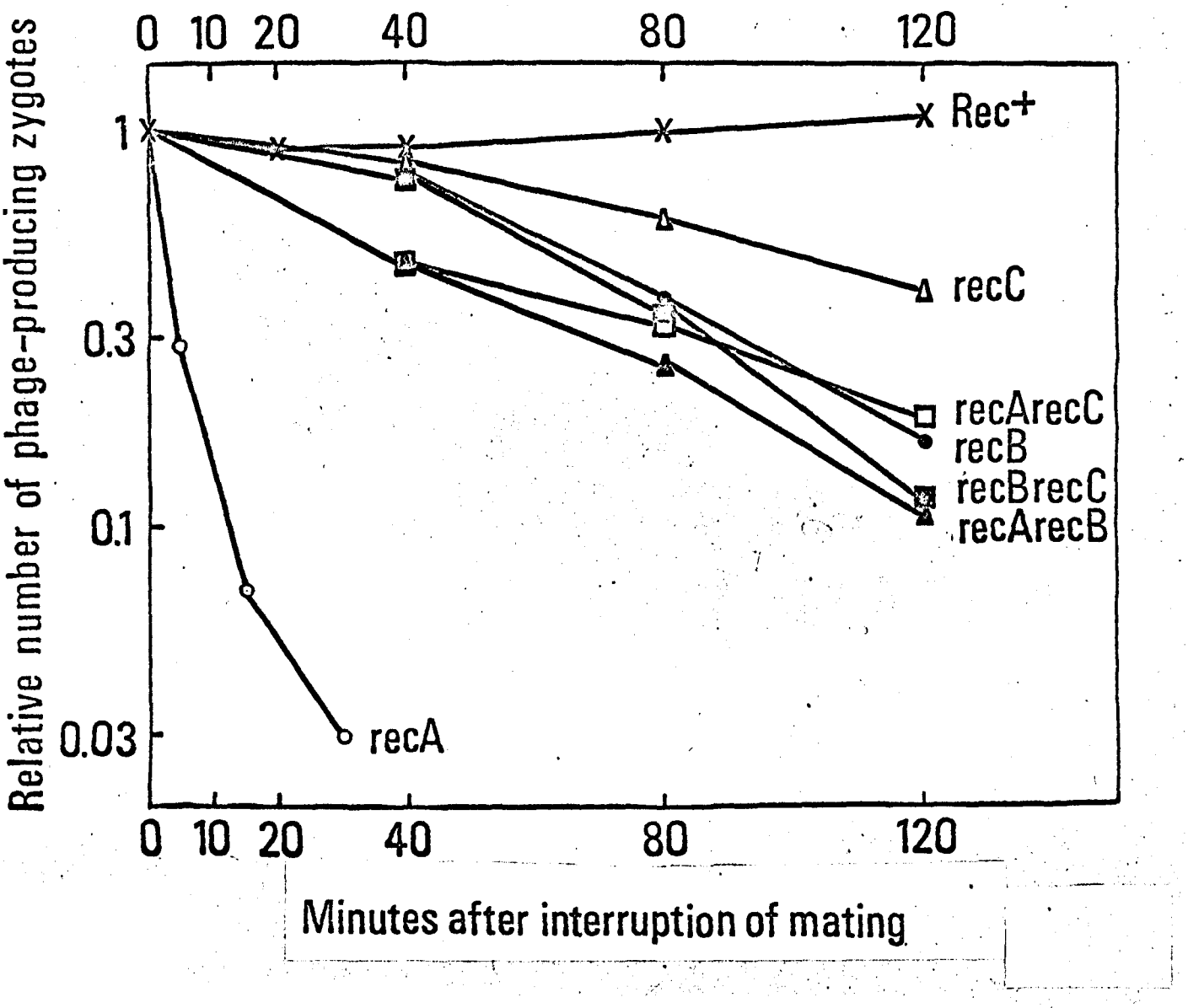


Figure 2

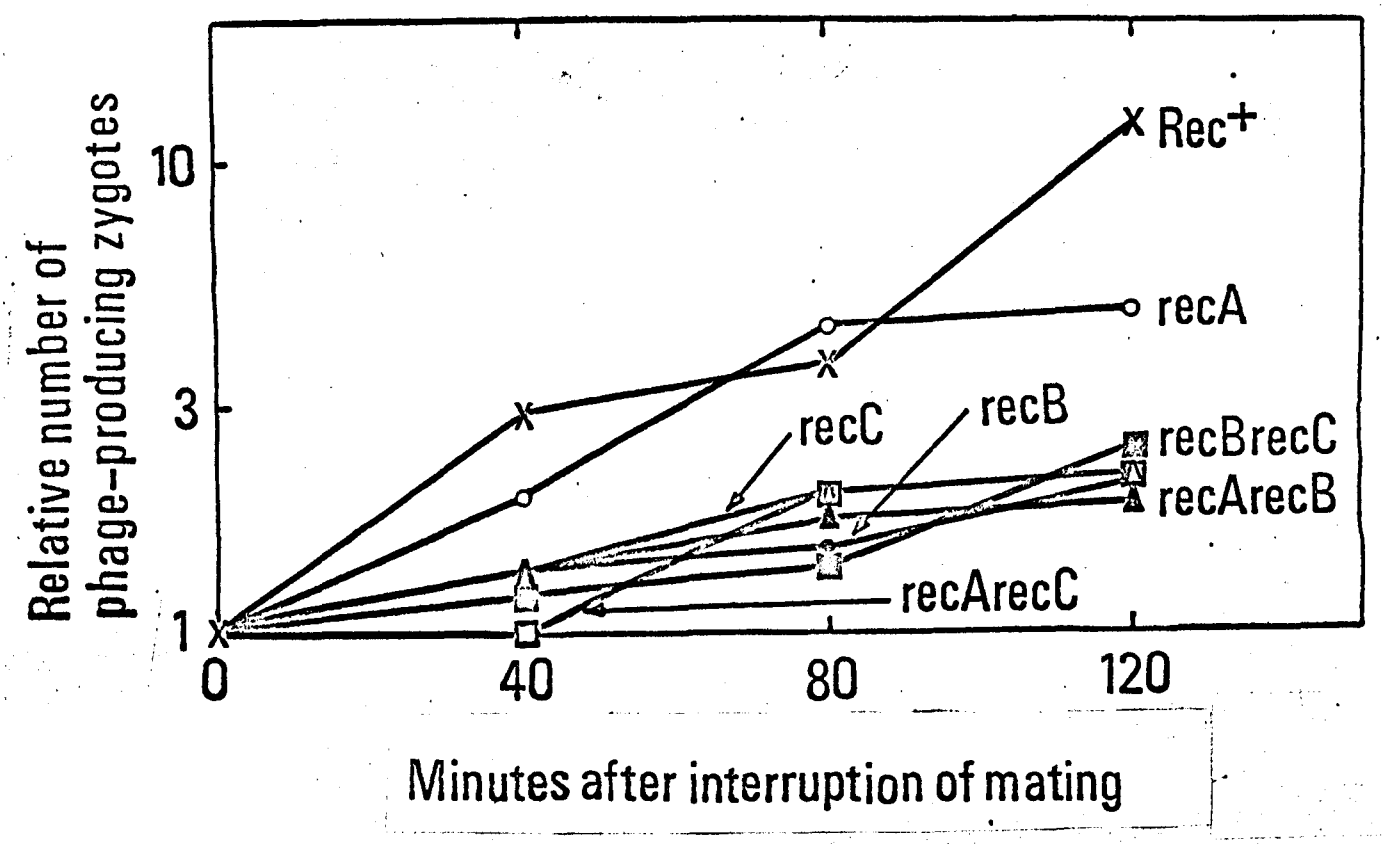

Relative number of phage-producing zygotes
10
3
1
0
40
80
120
Minutes after interruption of mating
Rec+
recA
recC
recB
recBrecC
recArecB
recArecC

Figure 3ε. 図17

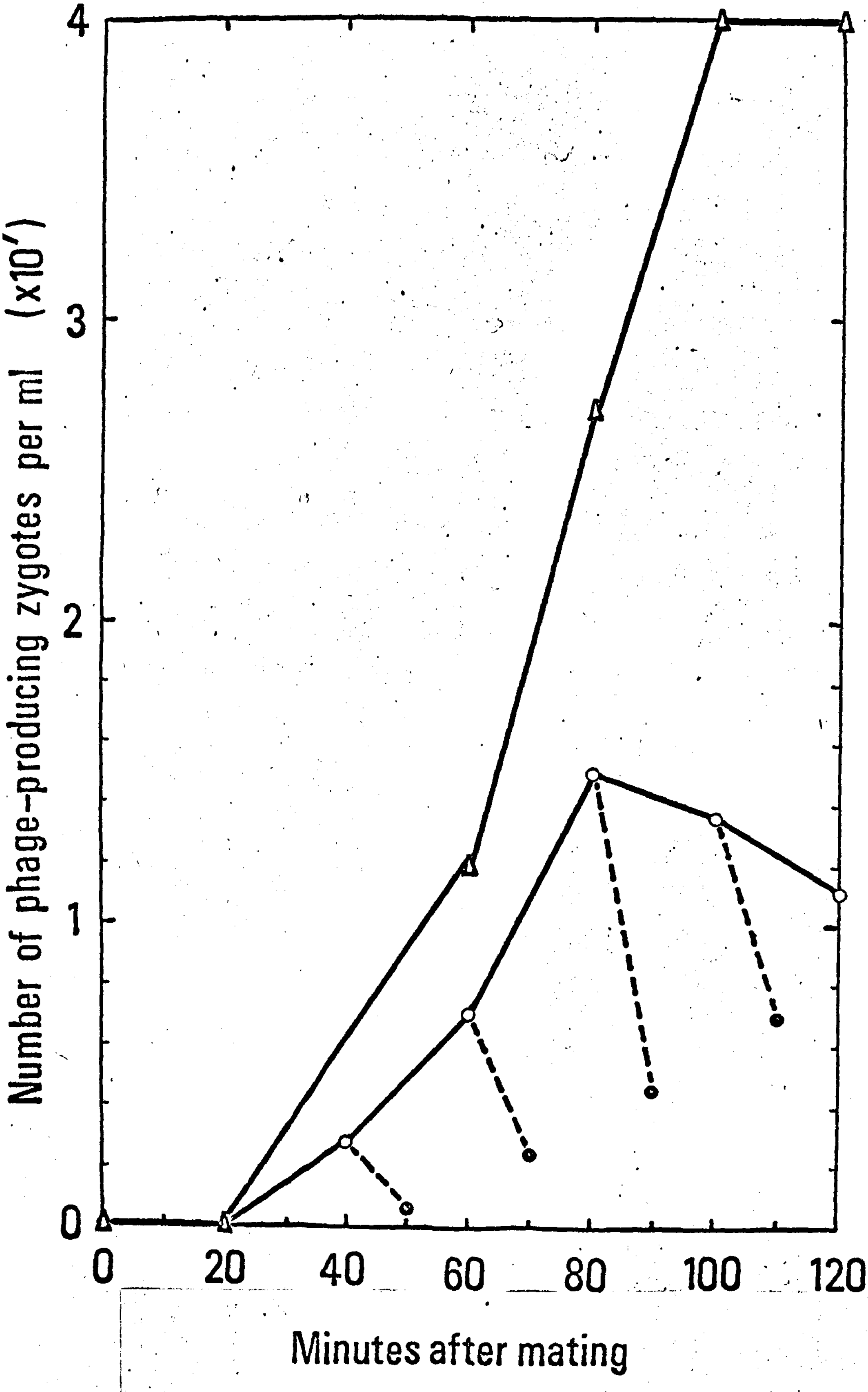
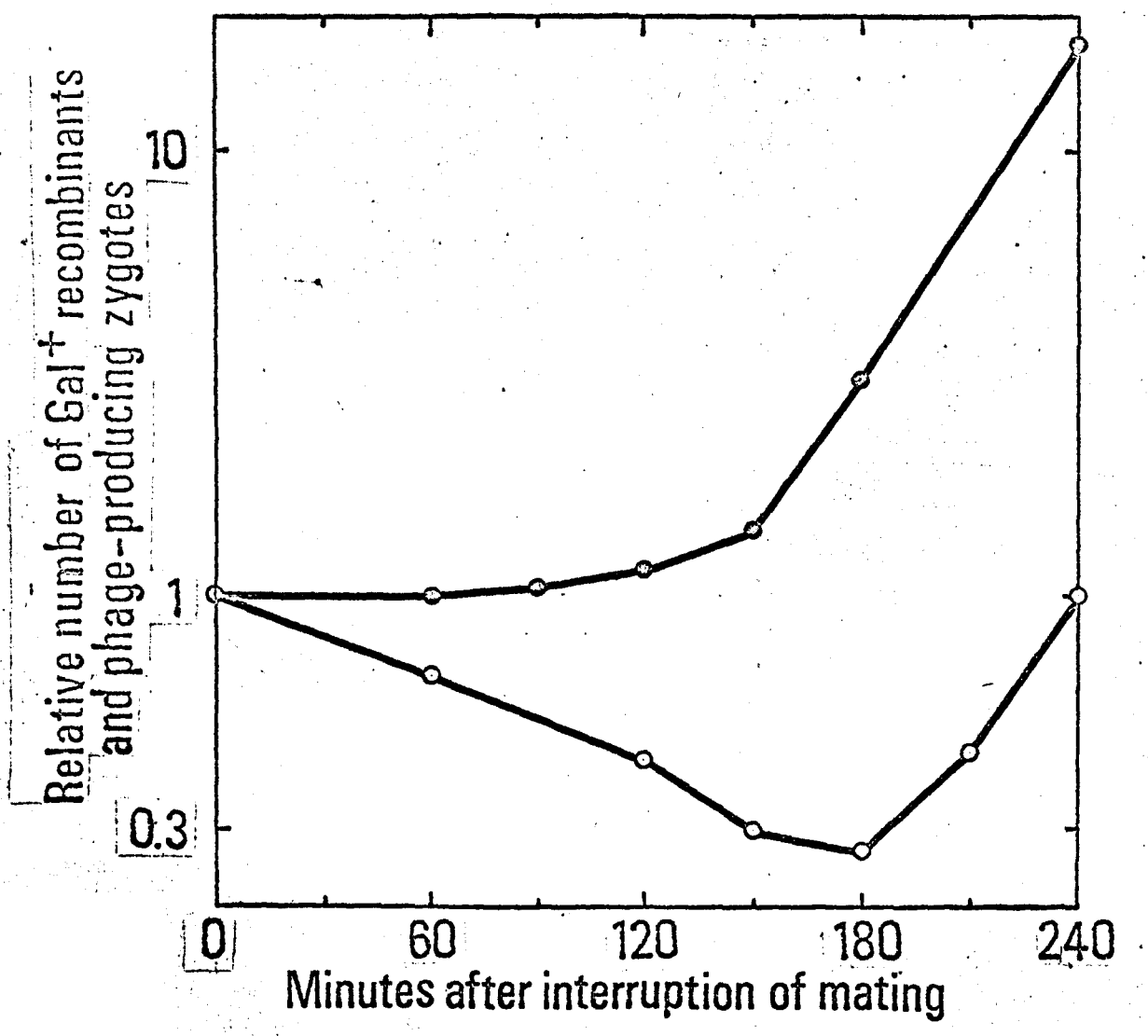


Figure 4


Relative number of Gal+ recombinants and phage-producing zygotes
10
1
0.3
0
60
120
180
240
Minutes after interruption of mating