MOLECULAR MECHANISMS OF GENETIC RECOMBINATION IN BACTERIOPHAGE LAMBDA : THE FORMATION OF COMPLEX GENOMES OF LAMBDA IN ABORTIVELY LYSOGENIC CELLS.

BY

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SUMMARY

In order to visualize genetically ~~in~~ the formation of complex genomes of phage λ in multiply infected Escherichia coli cells, we performed the segregation experiments as follows; When two strains of λb2 or λint carrying different sus markers, which are complementary with each other, are co-infected on non-permissive bacteria at a high multiplicity, the cells are able to produce phage particles upon induction, the number of which should decrease gradually with continued growth and then reach to a plateau only if two phage genomes are interacted before the segregation. The results of segregation experiments using the various combinations of rec, red and int mutants were that a considerable fraction of λb2 genomes, and presumably λ^+ too, interacted with each other by the bacterial Rec function, which were fairly stable without replication for long generations like as the ordinary abortive prophages of λb2. In order to study whether the interaction of infecting phage genomes in abortively lysogenic cells was the material (DNA) joining of two or more genomes or not, transduction of λb2 prophages with phage P1 were performed. The transduction of λb2 genomes is expected to be unsuccesful when the infecting phage genomes do not join with each other, since the length of DNA contained in transducing particles of P1 is 2.4 times that of λb2. ~~Their~~ These results show the formation of triploid-complexes, and presumably diploid-complexes too, of λb2 prophage genomes. Moreover the joining for complex genomes was demonstrated to occur not by end-to-end cohesion, but by the cross over between two circular λ DNAs from the transduction of λb2 prophage genomes by small transducing particles of P1 which can carry 95 % of λb2 DNA. As the burst derived from these complex prophages contained more recombinant phages than that prepared by the ordinary

phage crosses and showed the symmetrical marker distribution in the single burst experiment, we like to think that these complex genomes is the intermediate molecules produced by reciprocal material exchanges for the recombination in vegetative growth of λ. From these experimental results we discussed the model for general recombination of λ.

INTRODUCTION

Circularization of λ DNA *in vivo* may start with the cohesion occurring at its both ends by hydrogen bonding, and then the ends of each strand may be connected by ~~nucleotide~~ phospho diester bonds, resulting in the formation of twisted circular molecules. The cohesive ends of λ DNA are common among different molecules, or even with DNA of other related phages, such as ø80, 434, 21 *etc*, and the joining of two or more molecules by hydrogen bonds at their ends has been observed *in vitro* after appropriate thermal treatment (Hershey and Burgi, 1965; Yamagishi *et al*., 1965; Baldwin *et al*., 1966). Under these circumstances, one may predict the formation of complex genomes by end-to-end joining of λ DNAs occurring *in vivo* upon the infection of phages at a high multiplicity. The formation of complex genomes of phage λ may also take place *in vivo* in a different process, which is analogous to the prophage integration by the Campbell's model (1962). Namely , a single or odd number cross over occurring between two ring molecules of λ DNA would result in the formation of a diploid complex, either a ring or a rod depending on whether the exchange takes place in reciprocal way or not. Both of these processes for joining of λ DNA in bacterial cells are likely to happen.

In order to visualize the formation of complex genomes of λ, we took

the advantages of λ*b2* or λ*int* on one hand, which cannot be integrated into the host chromosome but persists as an abortive prophage (Kellenberger *et al.*, 1961a; Ogawa and Tomizawa, 1967), and the advantages of suppressor sensitive (*sus*) mutants of λ on the other, which can produce phage particles only on certain permissive bacteria (Campbell, 1961).

When the bacteria infected with λ*b2* or λ*int* at a high multiplicity of infection are incubated, the number of cells carrying abortive prophages first increases with bacterial growth, but gradually stops to increase, reaching a plateau which roughly corresponds to the multiplicity of infection. Hereafter, only one of the two daughter cells at each cell division receives an abortive prophage which neither is integrated into the host chromosome nor replicates, and non-lysogenic bacteria begin to segregate.

If two strains of λ*b2* or λ*int* carrying different *sus* markers, which are complementary with each other, are co-infected on non-permissive bacteria at a high multiplicity, the cells are able to produce phage particles upon induction. Abortive prophage genomes are transmitted unilinearly to the descendant cells and non-lysogenic cells segregated with cell multiplication. Further incubation should cause the gradual decrease of the number of phage-producing cells upon induction, since each abortive prophage of complementary pairs is separated into different cells at cell divisions. However, if two phages are interacted before the segregation, either they have joined together forming hetero-diploid complex genomes or have been recombined giving rise haploid *sus*$^+$ genomes, the cells among descendants inheriting such a unit of the abortive prophage should retain the ability to produce phage particles even after many generations. Whether a cell carries a hetero-diploid or a

sus$^+$- haploid can be determined by analysing the genotypes of phages released from a single cell (single burst experiment). The experiments performed in this line revealed that the joining of λb2 genomes takes place at an appreciable rate which is much higher than that of the formation of recombinant haploids.

Another line of evidence for the joining of λ genomes in vivo was obtained by utilizing a generalized transduction system of phage P1. It is generally accepted that each transducing particle carries one piece of genetic fragment derived from a continuous molecule of DNA. The molecular weight and the length of P1 DNA are 6×10^7 daltons and 35.5 μ, respectively, and its transducing particle also contains a genetic fragment of the same size without P1 DNA (Ikeda and Tomizawa, 1965a; Ikeda and Tomizawa, 1968). This is also true in the case of transducing particles carrying prophage λ, and therefore these particles must contain, in addition to λ genome, a small portion of bacterial chromosome adjacently connected to the integrated prophage, since λ DNA is about the half size of P1 DNA, 17.1 μ (Davidson and Szybalski, 1971). As λb2 cannot be integrated into the bacterial chromosome and there is a lower limitation in the size of DNA packed in a phage particle (Ikeda, personal communication), single λb2 genome is too small to be transducible, but the complex genomes of λb2 formed in multiply infected bacteria can be transducible. The joint transduction of two or three alleles of given λ genomes may be expected, as the size of a transducing fragment corresponds to about 2.4 folds that of λb2 DNA, and in turn, it would provide a good evidence for the joining of λb2 genomes occurring in vivo.

The transduction experiment by small particles of phage P1 are applicable

to clarify whether the hetero-complex genomes are produced by end-to-end cohesion or by recombination at another region. Transducing small particles of P1 contains bacterial DNA equivalent to 40 % of infective particles (Ikeda and Tomizawa, 1965b) or 95 % of λb2. As the terminal markers of susA and susR are cotransducible if λ DNA is circular, but an infective λ is not, the linkage of them can make proof of end-to-end joining or not. The dependency of joining of λb2 genomes to bacterial Rec, phage Red and Int functions was also studied by segregation experiments using the various combinations of rec, red and int mutants.

In this paper we described these experiments and their results that a considerable fraction of λb2 genomes jointed together by the bacterial Rec function in multiply infected cells, and discussed about the relation of joining of λb2 genomes to recombination mechanisms of phage λ.

MATERIALS AND METHODS

Nomenclature. Genetic symbols used to designate genotypes and phenotypes are those of Taylor and Trotter (1967).

Bacteria. Bacterial strains used were all derivatives of Escherichia coli K12. The permissive (sup^+) strains were C600 (Campbell, 1961) and its $tonA^+$ derivative C600S (Sato et al., 1968). The non-permissive (sup^-) strains were W3350 (Campbell, 1961) and its streptomycine-resistant mutant 594 (Weigle, 1966). C600S/P1 and W3350/P1 were P1-resistant mutants of C600S and W3350, respectively. 594A carrying recA41, 594B carrying recB21 and 594AB carrying recA41 and recB21 were recombination-defective derivatives of 594; these rec markers were introduced from W3623recA41 (Ogawa et al., 1968) and AB2470 carrying recB21 (Howard-Flanders and Theriot, 1966) by bacterial crosses and

P1-mediated transductions. Another recombination-deficient mutant W3623recA42 (Ogawa et al., 1968) and Kornberg's polymerase-less W3623polA were also used, which were kindly supplied by Dr. H. Ogawa. The selective indicator for the host-range (h) mutant of λ was CR63 (Appleyard et al., 1956), which is resistant to λh$^+$. Lysogenic strains were prepared with various phages as needed.

Phages. Phage strains used were λ$^+$, λimm^{434} (Kaiser and Jacob, 1957), λb2 (Kellenberger et al., 1961a), λb5 (immunity specificity to phage 21;Kellenberger et al., 1961b) and P1vir4 (produce small particles at a high frequency; Ikeda and Tomizawa, 1965a). The λ mutations used in various combinations were following: λsus nonsence mutations A11, E4, L63, P3 and R5 (Campbell, 1961); the cI mutations c60, and cI857 (Sussman and Jacob, 1962) which renders λ phage inducible by elevated temperature; tsP34 (obtained from Dr. J. Tomizawa), a mutation in the P gene which prevents plaque formation at 42° but not at 30° ; integration-defective int2 (Gingery and Echols, 1967) and vegetative recombination-defective redB114 (Echols and Gingery, 1968); and host-range mutation h.

Media. The following media were used: λ-broth and λ-agar (Matsushiro et al., 1964); EMB agar(Lederberg, 1947); Bacto-penassay broth (Difco); L-broth (Lennox, 1955); TM (10^{-2}M Tris-HCl buffer, pH 7.4, 10^{-3}M $MgCl_2$); λ-buffer (10^{-2}M $MgCl_2$, 0.002 % gelatin in TM); and anti-λ serum (prepared from rabbit immunized against purified λ phage particles and bacteria-agglutinating antibody in the serum was removed by adsorption with non-lysogenic bacterial cells).

λ phage lysate. Stock lysates; thermal induction was used to prepare stock

lysates of cI857 phages (Sussman and Jacob, 1962); UV-induction for cI^+ phages, in which 5 ml of λ-broth containing 2 x 10^8 cells/ml in a petri dish was exposed to a 15 w germicidal lamp (Toshiba) for 30 sec at a dose rate of 10 ergs/mm^2/sec; and infection of sensitive cells for λint2, λb2 and λc60. High titer lysates were prepared by vegetative growth in strain C600 or C600S, and the phage was purified through differential centrifugations. The high titer lysates were stored in suspensions of λ-buffer.

Phage cross. The recombinant phage strains needed were prepared by phage crosses, which were carried out by lytic growth at 37° after phage adsorption in 0.01 M $MgSO_4$ (20 min at 37°). A multiplicity of infection of 5 for each parental phage was used and at the end of the adsorption period the unadsorbed phages were inactivated with anti-λ serum. Then the infected cell suspension was diluted into λ-broth (1/100) and incubated for 90 min at 37°. After chloroform treatment the lysate was plated on an appropriate indicator bacteria for plaques.

Test for various genotypes of λ. In the construction of various recombinant phages, int and b2 markers were scored by the spot test of Gottesman and Yarmolinsky (1968) using EMB0 plate. The red character was scored by the ability of phage growth on W3623polA (Signer, 1971). Complementation tests for sus markers were performed as follows. After treatment of agar plates showing plaques with chloroform vapour, single plaques were picked with sterilized toothpicks and inoculated on an agar plate seeded with non-permissive bacteria by stabbing. Each stab was then covered with a tiny drop of phage suspension carrying an appropriate sus marker. The confluent lysis surrounding the stab after incubation indicated the presence of comp-

lementation of the testing phage against the sus phage applied. In the experiments in which two sus markers were involved, the stab inocula were arranged on quadruplicated plates, namely three plates with non-permissive lawn and one with permissive lawn; the stabbes on the latter were always made lastly, in order to confirm that the inoculation of phage on the former three plates was made satisfactory. Two of the three plates with non-permissive indicator were used for the complementation test for each sus markers involved. Phages able to grow on non-permissive cells without complementation were scored as sus$^+$, and that cannot grow either complementing phage were counted as the double sus mutants.

Segregation experiment of abortive λ prophages. An overnight culture of E. coli strain in λ-broth (3×10^9 cells/ml) was centrifuged, suspended in 0.02 M $MgSO_4$ at a concentration of 1.0×10^9 cells/ml and infected mixedly with two sus derivatives of λb2 or λint at a multiplicity of infection of about 5 for each. After adsorption for 15 to 20 min, the cell suspension was suitably diluted (at zero-time) in broth supplemented with anti-λ serum (K=1 to 2) and incubated at 37°. When the temperature-sensitive mutant, cI857, was used, the cells were incubated at 30°. Under these experimental conditions using fully grown cells with high multiplicity of infection, about 70 to 80 % of infected cells showed a lysogenic response, whereas at a low multiplicity of infection most of them show a lytic response. At intervals, the total number of cells in the culture and phage-producing cells after induction were measured. When the number of cells reached about 5×10^8 cells/ml, the culture was diluted ten-folds. The number of lysogenic or phage-producing cells was measured by plating with a permissive indicator bacteria after

induction by heating at 40° for 20 min or by UV irradiation.

Single burst experiment. The bacterial suspension was UV-irradiated for the prophage induction, and then highly diluted into broth and small samples transferred to each of a large series of broth tubes, in such a way that each tube had a low probability of receiving an inducing bacterium. The tubes were incubated for 120 min with aeration and the entire contents of each tube were then plated for plaques. An appropriate number of well isolated plaques were picked at random from each burst and tested for their sus characters by complementation tests.

Transduction of λb2 abortive prophage by phage P1. The fully grown bacteria in λ-broth were infected with λb2, washed by centrifugation and resuspended in L-broth containing anti-λ serum (K=1 to 2). The culture was incubated with shaking at 37° for at least 90 min, and then infected with P1 at a multiplicity of 3 after the addition of 0.0025 M $CaCl_2$. The culture was shaken for further 120 min, and a small amout of chloroform was added. The lysate thus obtained was used directly for transduction experiments, after the removal of cell debris by a low speed centrifugation. Absence of viable bacteria as well as of λ phage particles in the P1 lysate was checked before using it.

The recipient bacteria in transduction experiments were prepared by the following way: bacteria grown in L-broth to a concentration of 4×10^8 cells/ml were collected by a centrifugation, suspended in Tris-HCl buffer (pH 7.2) containing 0.0025 M $CaCl_2$, and then irradiated with UV light. The recipient cells were infected with P1 at a low multiplicity of infection (less than 0.1) and incubated for 30 min. The mixture were then plated with

an indicator of C600S/P1 or W3350/P1 for λ plaques.

When the transduction by small particles of phage P1, the P1 lysate purified by a zone centrifugation was used as donor.

Zone centrifugation of phage P1 particles. Zone centrifugation of phage particles was carried out in a density-gradient of sucrose, 5 % to 20 %. A 0.1 ml sample was gently layered on 4.5 ml sucrose-gradient and centrifuged at 12° in a Spinco SW39L rotor for 45 min. Fractions were collected from the bottom of the tube (Ikeda and Tomizawa, 1965a).

RESULTS

Segregation of plaque-formable cells in permissive strain infected with λb2cI857. A typical result of segregation experiment with λb2cI857 is presented in Fig. 1. The lysogenized cells started to grow after a short lag. At about four to six hours after infection, the number of phage-producing bacteria, after thermo-induction, reached a plateau, and non-lysogenic bacteria began to segregate. The ratio of the number of bacteria carrying λb2cI857 at the plateau to that of the colony formers immediately after the infection was roughly equall to the multiplicity of infection. These results are essentially the same as those reported by Kellenberger et al. (1961a) and suggest that the infected phage becomes an abortive prophage without appreciable multiplication and that the prophage λb2cI857 is unilinearly transmitted to the descendant cells. Later, however, Ogawa and Tomizawa (1967) demonstrated that multiplication of phage genomes may occur before establishment of abortive lysogenization by infection of λb2 under the simillar condition; though the extent of multiplication vary in different

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experiments, usually about half of infected phage DNA may duplicate. If the phage genomes separate completely from each other by cell division, a greater final number of abortive lysogenic cells would be expected. Thus the phenomenon, that the number of abortive lysogenic cells at the plateau compared with that of colony formers immediately after infection was similar to the multiplicity of infection, suggested the incomplete separation of abortive prophage genomes, such as the formation of dimer genomes, at cell divisions.

Segregation of plaque-formable cells in non-permissive strain co-infected with two sus derivatives of λb2. The association of two or more phage genomes was visualized in the following experiment. The culture of W3350 (sup$^-$) was co-infected with λb2susA11 and λb2susP3 at a multiplicity of infection of five for each and usual segregation experiment was performed (Fig. 2). If the infected phage genomes are completely independent with each other in abortively lysogenic bacteria, the number of phage-producing cells after induction is expected to increase for a short time and then gradually to decrease. As can be seen in Fig. 2, however, the number of phage-producing cells increased for 90 minutes, decreased gradually and reached a plateau at the level of about 70 % of the colony formers immediately after the infection. This plateau level was much higher than the background level infected with λb2susA11 alone or λb2susP3 alone under comparable conditions.

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Similar observations were also made with other combinations of sus markers, such as λb2susA11 and λb2susR5, λb2susP3 and λb2susR5, or λcI857int2-susE4 and λcI857int2susR5 (data not described). In all cases the plateau levels of phage-producing cells of W3350 were more or less comparable under

the uniform condition regardless of the genetic distance of two markers involved.

Were the prophages giving the plateau of the number of phage-producing cells produced only after or during DNA replication of λ? To answer this question, the following segregation experiments were performed. 594 (sup⁻) was infected with λb2susE4tsP34 and λb2susL63tsP34. After adsorption at 42°, the culture was diluted and divided into two portions. One was incubated at 30° and the other at 42°. If the prophage giving the plateau is produced only after or during DNA replication, the level of plateau at 42° is expected to be drastically reduced than that at 30°. But the results obtained (Fig. 3) showed that the level of plateau at 42° was slightly lower than that at 30°. Thus DNA replication of λ was not necessary for the plateau formation of the number of phage-producing cells.

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These experiments revealed that a considerable fraction of mixedly infected λb2sus or λintsus phages interacted with each other, resulting in the formation of abortive prophages which are capable of producing phage particles in non-permissive host bacteria even after segregation. It is evident that the interaction was mostly occurred within first one or two cell generations. The resultant products of interaction are expected to be either hetero di- or more-ploids carrying two sets of phage genomes derived from both parental types, or recombinant haploids having sus⁺ genotype. Since the plateau level of phage-producing cells is independent of the genetic distance of two markers involved, the former case is more possible. In order to distinguish these products, the following single burst experiment was performed.

Single burst experiment of lysogens carrying complex genomes of λb2. Non-permissive bacteria of W3350 were mixedly infected with λb2susA11 and λb2susP3 at a multiplicity of five for each as described above, and incubated for 240 minutes in order to distribute each unit of segregation of λ abortive prophages into different cells among descendants. The culture was then UV-irradiated, diluted in broth and divided into 150 tubes, aiming at 0.2 phage-producing cells per tube. At the same time, an aliquot was plated to measure the number of phage-producing cells in the UV-irradiated culture. In this experiment, the actual number of phage-producing cells per tube was turned out to be 0.24 in average. After 120 minutes' incubation to let the induced cells to lyse, the entire content of each tube was plated with indicator bacteria of C600S for plaques. Among the 150 tubes, 116 (77.3 %) showed no plaques while the rest, 34 tubes, formed many plaques. According to a Poisson distribution, 77.3 % of zero-plaque fraction is expected if the each original tube contained 0.255 phage-producing cells in average, showing a good agreement with measured value of 0.24. Thus in the most of tubes in which phages were produced, the phages were derived from the burst of a single bacterium. The probability expected for a single burst was 87 %, or 29 out of 34 tubes. A dozen or two dozens plaques from each burst were randomly picked and tested for their sus characters by complementation. The results obtained with 22 bursts are summarized in Table 1. 17 out of 22 bursts showed the presence of both alleles of two loci, namely $susA^+$, susA11, $susP^+$ and susP3, and 4 out of 22 contained at least three out of four genes. Only one burst seemed to contain a pure genotype of $susA^+susP^+$. Thus it was revealed that the majority of phage-producing cells after prolonged incubation still carries

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two parental genotypes as if they are connected with each other forming a stable unit for segregation.

As showen in Table 1, many of the single burst analyzed contained two major types of phage with more or less comparable frequency; most of them contained both parental types as the major class in a burst , but some had two reciprocal recombinant types, susA$^+$susP$^+$ and susA11susP3. These results seem to indicate that the phage growth after induction tends to start with two complementary phage genotypes in each cell, and during the lytic cycle some recombinant types between them were also formed which are seen as a minority components in each burst.

In order to see the extent of recombination during the phage growth, the following single burst experiment was made. To make a direct comparison to the above results, the experiment was performed with the cells of W3350 carrying one of each abortive prophages of λb2susA11 and λb2susP3 within a cell but independently. Such bacteria were prepared as followes; W3350 was infected with λb2susP3 at a multiplicity of five and incubated for 180 minutes to let the abortive prophages to segregate among the descendants, and then infected with λb2susA11 at a low multiplicity of 0.01. Under these conditions, most of cells which can produce phage particles upon induction were expected to be carrying one copy of each phage existing independently with each other. The single burst experiment was carried out just after the second phage infection in the same way as described above and the results obtained were presented in Table 2. In all the burst tested except one (14/15), the both parental types were major and recombinant classes were only occationally seen. No burst contained the recombinant types as the major class. These

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results were in turn interpreted as the indication for the interactions occurring between two phage genomes before the phage induction in the single burst experiment shown in Table 1.

Transduction of λb2 abortive prophage by phage P1. Although the interaction of infected phage genomes in abortively lysogenic cells was demonstrated above, there is no evidence indicating the joining of two genomes at DNA level. To clarify this point, the transduction experiment of λb2 abortive prophages mediated by phage P1 was performed.

Ikeda and Tomizawa (1965a) demonstrated that the transducing particles of phage P1 contained the almost equal amount of DNA with infective particles and that most of the transducing particles lack phage genome and carry only fragments of the bacterial chromosome existed at the time of infection. If the above results are applicable to the case of transduction of λb2 abortive prophage genomes, the transduction is expected to be unsuccessful when the infecting phage genomes did not join with each other, since the length of P1 DNA is 2.4 times that of λb2 (Ikeda and Tomizawa, 1968; Davidson and Szybalski, 1971).

The culture of C600S was mixedly infected with λb2susA11 and λb2susP3 at a multiplicity of five for each, washed and resuspended in L-broth containing anti-λ serum at three different cell densities, namely 10^8, 10^7 and 10^6 cells/ml. The cultures were incubated with shaking at 37° for 60, 150 and 240 minutes, respectively, before the infection of P1. The cultures were continued to be incubated until lysis. The titer of P1 and of λ-transducing particles of these three lysates were summarized in Table 3. Since the 60 minutes' lysate gave a poor yield of P1 as well as the transducing particles

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by unknown reasons, the other lysates were used for further studies.

As shown in Table 3, λb2 can be transduced with a frequency of 10^{-4} per initially infected λb2, which is somewhat lower than that of integrated prophage from lysogenic bacteria (10^{-3}) under the condition used. These transducing particles of λb2 were most likely to be derived from its abortive prophages and not from vegetative phages, since the lysis of cells by original infection of λb2 may be completed at about 120 minutes. In addition, our preliminary experiment showed that the growth of P1 is inhibited in the cells in which phage λ have been growing vegetatively. The density of transducing particles measured in a CsCl density-gradient centrifugation was the same as that of ordinary transducing particles, and therefore they contained DNA much larger than the single genome of λb2 (Dr. H. Ikeda, personal communication). The presence of transduction of λb2 was thus already indicative for the joining of λb2 genomes, which was confirmed by the single burst experiment made with the transduced cells (Table 4). A considerable fraction of transductants (14/47) produced more than two types of phages, and thus the formation of hetero-complex genomes of λb2 was evident. In Table 4, single burst from which only one or the other parental types was detected were also seen; 12/47 for susP3susA$^+$ and 19/47 for susP$^+$susA11. Since, however, we tested only twelve plaques from a burst, some of those single bursts possibly contain other genotypes as a minority class. These results are consistent with the conclusion reached in the segregation experiments, and also revealed that the joining of λb2 takes place not only in non-permissive cells, but also permissive cells, which was not demonstrated in the segregation experiment.

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There seems a marked difference between two results of single burst

experiments presented in Table 1 and 4. In the case of transductants, although more than two types of phages were found from a single burst, only one of those was predominantly contained, while in the case of the segregation experiments, two complementary genotypes were together making the major class in a burst. In the former, most of the burst contained three out of four markers, susA$^+$, susA11, susP$^+$ and susP3, while in the latter, all of these four markers were usually detected from each single burst. These difference may be due to the different structures of complex genomes of λb2 involved which will be discussed later.

Evidences for the formation of triploid complex. As mentioned above, the transducing particles of P1 contain DNA equivalent to 2.4 folds of λb2 DNA, and transductants of λb2 prophages should be derived from tri- or more-ploid of λb2 genomes. To confirm the existence of trimer directly, three strains of λb2 carrying different alleles of the immunity locus, namely imm$^{\lambda c^+}$, imm$^{\lambda c}$ and imm^{21}, were mixedly infected on C600S at a multiplicity of five for each, and P1 lysate was prepared from these cells by the procedures described above. Transduction was made with C600S as the recipient and C600S/P1 as the indicator for the λ plaques transduced. After sterilizing the plates with chloroform vapour, several hundreds of well isolated plaques were randomly picked and suspended in a small amount of buffer separately. The phage suspensions thus obtained were spotted on the lawns of C600S(λ) and C600S(λb5) for testing their immunity characters, on which imm$^{\lambda c^+}$ and imm$^{\lambda c}$ were easily distinguishable by their plaque morphology. The results obtained are summarized in Table 5. Among the transductant plaques tested, 6 (1.6 %) of a single transduction event which contained three different types of phages in respect of

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immunity locus is lower than that expected. To detect all of these three alleles in a single transductant plaque the following various conditions must be fulfilled. (1) three types of λb2 must be infected together, (2) three types must be joined together, (3) three imm genes must be included in a transducing particles; if a transducing fragment of 2.4 folds length was randomly cut out from a three length circle, the chance would be about 0.4, (4) all three markers introduced into a recipient cell by a transducing particle must appear in phage particles produced in it, and (5) these three types of phages must grow together during the formation of plaques in an appreciable extent, so that all of them detectable in the final check. After considering those various probabilities, the observed value of 1.6 % may be in the order of expected one, and this in turn suggests that all the λb2-transducing particles may be derived from triploid complexes rather than diploid ones. The values observed on transductants having only single type of imm gene were unexpectedly high, which might be reflecting the fact that λb2 genomes do multiply in a certain extent before establishing the abortively lysogenic state (Ogawa and Tomizawa, 1967). If such replication does not uniformly occur on every phages in a mixedly infected cell, one type of them tend to be enriched, increasing the chance to form homo-complexes and the same time decreasing the chance for hetero-complexes. Anyway, the existence of co-transduction of three alleles of a gene strongly indicates the formation of tri-parental complex genomes at DNA level.

A suggestive evidence for the triploid complexes has also been obtained in the segregation system of abortive prophages. In order to detect such complexes selectively, three double sus mutants of λb2, namely λb2susA11susP3,

λb2susP3susR5 and λb2susR5susA11, were mixedly infected on non-permissive W3350. The following procedures were same as in Fig. 2. The cells which can act as plaque-centers must carry susA$^+$, susP$^+$ and susR$^+$ together, each of which must be originated from three different parents. The number of phage-producing cells was first increased and then decreased toward to a plateau after several generations. The plateau level observed (data not shown) was considerably lower than that observed in Fig. 2. The occurrence of triploid complexes was thus suggested and the plateau level observed only served to indicate the maximum frequency of the formation of hetero-complexes.

Transduction of λb2 abortive prophage by small particle of phage P1. Did the joining of prophage genomes in abortively lysogenic cells occur by end-to-end joining at cohesive ends or by recombination at another region? To answer this question, the following experiments were performed. Transducing small particles of phage P1 contained bacterial DNA equivalent to 40 % of DNA in infective or normal transducing particles and could transduce bacterial markers as well (Ikeda and Tomizawa, 1965b). Accordingly P1 small particles can transduce only 95 % of a single λb2 genome, and therefore these particles cannot form λ plaques by a single infection. Transduction of λ genes must be detected by marker rescue method.

Insidentally, during the course of this investigation, we have obtained an interesting result that a small transducing particle of P1 could not carry complete λb2 DNA (106 % of P1 small DNA), but could carry complete λb2b5 DNA (101 % of P1 small DNA) which has infectivity by a sngle infection. The densities of small particles carrying defective λb2 DNA and infective λb2b5 DNA in a CsCl solution were 1.428 and 1.431 g/cm^3, respectively.

As the ~~preliminary~~ control experiment, the transduction with small particles of P1 was performed in which the integrated prophage or monomer abortive prophage was used as donor. W3623recA42(λh), in which the interaction of infected λ genome with prophage one was inhibited, was infected with λc60 at a multiplicity of infection of 2. After 100 minutes' incubation, the culture was infected with phage P1 and led to be lysed. As the yield of P1 on recA strain was very poor, 50 ml of lysate was concentrated, followed by a sucrose density-gradient centrifugation to purify small particles. Transduction experiments were performed, in which recipients were W3350, W3350(λimm^{434}), W3350(λimm^{434}h), W3350(λimm^{434}susA11), W3350(λimm^{434}susR5) or W3350(λimm^{434}-susA11susR5), and assayed for cI^{+} and cI on W3350(λimm^{434}susA11susR5)/P1 (Table6). T-6
Cotransduction of susA^{+} and susR^{+} markers occurred at about equal frequency for cI^{+} and cI. On the other hand, cotransduction of cI and h markers was observed only when donor was the monomer abortive prophage but not the integrated prophage. Thus the near markers could be transduced by P1 small particles under the condition used. It was also demonstrated genetically that the abortively lysogenic genome was circular.

The culture of C600S was mixedly infected with λb2c60susR5 and λb2hsusA11 at a multiplicity of five for each, washed, and resuspended in L-broth containing anti-λ serum with shaking at 37° for 120 minutes before the infection of P1, followed by further incubation until lysis. P1 small particles purified by zone centrifugation was used for transduction of the imm$^{\lambda}$ gene. Recipients used were W3350, W3350(λimm^{434}), W3350(λimm^{434}susR5), W3350(λimm^{434}susA11) and W3350(λimm^{434}susA11susR5) after UV irradiation. imm$^{\lambda}$ transductants on the indicator of W3350(λimm^{434}susA11susR5)/P1 were measured for cI^{+} and cI

markers and their results were shown in Table 7. Most of the transductants carrying both parental markers seemed to be derived from recombinant dimer DNA molecules in which cross over occurred between susA and h, h and cI, or cI and susR. For example, $imm^{\lambda c}h$ transductants on W3350(λimm^{434}susA11) were expected to be produced if the cross over for dimer formation occurred between susA and h, or h and cI, $imm^{\lambda c}h$ transductants on W3350(λimm^{434}susR5) must be produced by cross over between h and cI, or cI and susR on two paretal genomes, and so on. Therefore the end-to-end cohesion is not only the mechanism for dimer formation, but cross over must be considered.

T-7

What functions are concerned about the joining of infecting phage genomes? The joining of infecting phage genomes occurred at the region out of the cohesive ends as shown above. Accordingly recombination was probably related to this joining. Bacterial Rec, phage Red and Int had been known as the recombination function. To test which recombination function(s) are concerned about the joining of infecting phage genomes, the various combinations of rec, red and int mutants were used for segregation experiments; (A) $Rec^-Red^-Int^-$ system, 594A co-infected with λcI857int2redB114susE4 and λcI857int2redB114susR5, (B) $Rec^+Red^+Int^-$ system, 594 co-infected with λcI857int2susE4 and λcI857int2susP3, (C) $Rec^+Red^-Int^-$ system, 594 co-infected with the same combination as (A), (D) $Rec^-Red^+Int^-$ system, 594A co-infected with the same combination as (B), (E) $Rec^-Red^-Int^+$ system, 594A co-infected with λb2cI857redB114susL63 and λb2cI857redB114susP3, and (F) $Rec^-Red^-Int^+$ system, 594A co-infected with λb2cI857redB114susL63 and λb2cI857redB114susE4. As described in materials and methods, the number of total cells and phage-producing cells were measured at intervals. Results obtained were shown in Fig. 4-(A) to (F), and could

F-4

be divided into two groups; one gave the plateau of phage-producing cells such as Fig. 2 and the other did not. The prophage giving the plateau of the number of phage-producing cells was produced only in Rec^+ cells, but not in Rec^-. Thus the prophage giving the plateau was probably made by Rec function. But there was still the following possibility. Some unknown function other than Rec might produce the joint prophages, which would be degraded from the end after non-reciprocal recombination event in Rec^- as UV-irradiated DNA of recA bacteria (Howard-Flanders et al., 1966) or as transferred DNA from Hfr to a recA recipient (Itoh and Tomizawa, 1971). As such DNA degradation (inactivation) was inhibited by introduction of recB mutation into recA cells, segregation experiments in $Rec^-Red^+Int^-$ system and $Rec^-Red^-Int^+$ system were performed by use of 594AB instead of 594A. The results obtained were similar to those in the case of 594A and the plateau could not observed. Moreover, when 594B was co-infected with the same combination of λ as (A), the plateau of phage-producing cells was produced considerably at lower level than that in (A) (data not shown). Thus the plateau observed here was surely produced by RecA function.

DISCUSSION

The results presented herein lead to the conclusion that the joining of λb2 genomes takes place in multiply infected bacteria, and the resultant polymer-complexes are fairly stable, being maintained for many generations without replication like as the ordinary abortive prophages of λb2. The occurrence of complex genomes is rather frequent under our experimental conditions. In the experiment described in Fig. 2 about one hetro-diploid

was formed per bacterium on which five copies of each parental phages were infected in average. Considering about homo-diploids which were not detectable in our system, two dimers were formed by using four out of ten copies introduced. This efficiency of joining is too high to be believable, and also contradicts to the fact that the total segregation level of λb2 abortive lysogens is more or less equal to the number of λb2 phages applied. However, we must recall the finding by Ogawa and Tomizawa (1967) that λb2 does multiply in a certain extent before establishing the abortive lysogeny. Accordingly the input multiplicity of infection does not directly indicate the number of phage genomes per cell, although the extent of limited growth had not been measured in our system. For simplicity, if every phage genome duplicate once and then stop to grow, ten infected copies in a cell would become twenty and two dimers would be formed by using four out of twenty. This calculation may be too simple, but the situation reached is more or less close to the observed one.

Two possible mechanisms for the joining of two phage genomes have been considered in the introduction of this parper; end-to-end cohesion and cross over joining. The complex genomes formed by these two mechanisms are schematically illustrated in Fig. 5. The possibility of end-to-end joining seems to be unlikely from (1) the single burst experiment of complex prophages (Table 1), (2) P1 transduction of complex genomes by small particles (Table 7) and (3) the dependency of formation of complex genomes to bacterial Rec function (Fig. 4). Thus the cross over joining is more reasonable and , in this case, two DNAs, or at least one of them, must be circular molecules, ~~unless~~ otherwise the resultants of cross over do not become complex, but two monomer re-

F-5

combinants. Upon induction of these dimer prophages to vegetative growth, either (1) they may grow as dimer and reduced to monomers on their maturation process to phage particles or (2) they may reduce to two monomers before the replication. In both cases the reduction of dimers to monomer genomes at the phage maturation process must be occurred by cutting at a fixed position for cohesive ends, because the genetic map of vegetative λ is linear and the linkage of terminal markers of susA and susR has not been observed. In many single bursts in Table 1, two major classes having symmetrical genotypes (either $susA^+susP3$ and $susA11susP^+$, or $susA^+susP^+$ and susA11susP3) in a burst were observed. In these cases a single cross over event to form a dimer seems to have the mechanism of reciprocal exchange between two DNA molecules; that is to say, a cross over involves two concomitant process of breakage and re-union. By this mechanism a cross over between two circular DNA molecules eventually results in the formation of a dimer, presumably circular (see below), molecule; or reversely from a dimer circular DNA, two monomer circular DNAs are eventually formed. Similar observations have been described by Herman (1966) and Meselson (1967); When F' factor was integrated into and then excised from the bacterial chromosome, there were reciprocal exchanges between the markers on F' and on bacterial chromosome. This was presumably mostly dependent on bacterial Rec function.

In contrast to the symmetrical appearence of the phages in single bursts in Table 1, some distortions of the distribution of four markers, namely $susA^+$, susA11, $susP^+$ and susP3, are observed as the general feature in the single bursts derived from P1 transductants (Table 4). This probably reflects what is derived by phage P1. That is a linear DNA molecules having 2.4 equivalent

of λb2 genome, derived from two parental types, and the ends of complex molecules are presumably at random being cutted from trimers. On the induction of λ from such a complex molecule, one may expect uneven appearance for four markers among the progeny particles. This is in turn suggests a symmetrical structure of complex prophages in abortive lysogens studied here.

Reciprocal cross over between two circular DNA molecules of phage and bacteria has been implied in the model of prophage integration by Campbell (1962), and our results suggests a comparable process also occur between two phage DNA molecules. If the dimer formation occurred in multiply infected cells prior to the prophage integration, it eventually form a double lysogen.

What is the biological role of the complex genomes observed? The frequency of the formation of dimers in segregation experiments was roughly comparable to the recombination frequency between two terminal markers in ordinary phage cross. The recombination frequency observed in the lysate derived from these heterodimers was greater than that in ordinary phage crosses. For example, the recombination frequency between susA and susP markers in both cases were 25 % (calculated from burst samples described in Table 1) and 11 %, respectively. We like to think the dimer complexes observed here as the intermediate products of recombination of phage λ in vegetative growth. At the phage maturation process, the dimers are then reduced to monomer genomes, being cutted at a fixed position for cohesive ends, and thus giving rise to recombinants of single cross over type. The genetic map attained in this way is linear, as if the recombination occurred between two linear molecules. The reduction of a dimer to monomers may also occur by cross over at homologous regions rather than at cohesive ends, and this gives the recombinants of double cross over type.

But its frequency should be very infrequent than that of reduction by cutting at cohesive ends, or the genetic map of λ is unable to be linear. Moreover, as the dimer prophages were very stable for long generations shown in Fig. 2, the reduction of dimer to monomers was reppressed in the lysogenic state, which indicates the reduction to be independent of bacterial Rec function. If the function responsible to the reduction process of dimer is also involved in the maturation process, in which the cutting of circular λ DNA at cohesive ends occur and the linear DNA is packed into the head structure, the replication of dimer DNA must first happen and then reduction occurs. For the maturation process presumably occur in the last stage of phage deveropment after induction (Dove and Weigle, 1965; Kaiser and Inman, 1965). From these considerations, it seems that the recombination of λ by Rec occur through complex genomes as an intermediate which replicates by itself before maturation. If this intermediate is produced by cross over between two markers on each parental genomes, the two recombinant phages having symmetrical genotypes with each other must be produced. On the other hand, if it is produced by cross over at either side of two markers, the original two parental types without recombinants must be produced.

Kellenberger (1971) described the similar model for recombination of λ suggested from the recombination experiment between λ*b2b5* and λ*dv*.

Though the complex genomes were produced only by Rec function in our experimental system, it was unknown whether Int and Red functions could also produce the complex genomes. For example, the amount of *int* and *red* products in these abortively lysogenic cells were too little to perform ordinary recombination. Genes *O* and *P* were enough transcribed, since the replication of infected

λ DNA occurred considerably before establishing the abortive lysogeny (Ogawa and Tomizawa, 1967). Accordingly the left operon from the N gene must be transcribed in these cells (Echols, 1971). Moreover, as the integration process (if λb2$^+$ or λint$^+$) occurred only under these conditions and int and red were co-transcribed (Echols, 1971), the amount of int and red products were presumably enough to promote recombination. In turn, it was suggested that Int and Red functions could not produce complex genomes and , therefore, the recombination by Int and Red was promoted through another intermediate molecules. However another possibility for Int was considered; as the ratio of recombination frequency by Int between λb2 x λb2, λb2 x λb2$^+$ and λb2$^+$ x λb2$^+$ was 3 : 12 : 2 (Echols et al., 1968) or 2 : 44 : 7 (Weil and Signer, 1968), and we used λb2 to test for Int in segregation experiments, the Int could not functionate to produce complex genomes by the reason of substrate defect but not by the reason of less amount of int products.

ACKNOWLEDGEMENTS

The auther wishes to express his heartiest thanks to Drs. Jun-ichi Tomizawa, Haruo Ozeki, Aizo Matsushiro, Noriko Ikeda, Hideo Ikeda and members of the laboratory of genetics for their encouragement and invaluable advices and discussions.

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Table 1. Single burst experiment after segregation of λb2 abortive prophages.[a]

Genotype			
susA$^+$susP$^+$	susA11susP3	susA$^+$susP3	susA11susP$^+$
0	0	6	6
0	0	6	18
0	0	4	8
0	0	1	11
0	1	7	5
0	2	7	3
1	0	5	6
3	0	4	5
4	0	7	1
6	0	1	17
1	1	9	1
1	1	1	9
2	2	6	2
6	5	0	1
5	4	1	2
9	0	1	2
2	4	5	1
8	0	16	0
6	0	0	18
3	0	0	9
1	0	0	19
24	0	0	0

a sup$^-$ bacteria of W3350 were co-infected with λb2susA11 and λb2susP3 at a multiplicity of 5 for each and incubated in broth supplemented with anti-λ serum at 37° for 240 min, at which time single burst experiment was performed. The average number of phage-producing cells per tube was 0.255 and 34 bursts samples were obtained. Among them, 22 burst samples were used for further

study. Adozen or two dozens plaques from each burst were randomly picked and tested for their *sus* characters by complementation. The contents of genotypes in each burst were described on each line of table. When summing up these samples, the recombination frequency, (*susA*$^+$*susP*$^+$ + *susA11susP3*)/(*susA11susP*$^+$ + *susA*$^+$*susP3*), was calculated to be 30.6 %.

Table 2. Single burst experiment using non-permissive cells carrying one of each abortive prophages of λb2susA11 and λb2susP3 within a cell but independently. [a]

Genotype			
susA$^+$susP$^+$	susA11susP3	susA$^+$susP3	susA11susP$^+$
1	0	10	8
2	1	10	9
0	0	10	11
0	0	13	10
0	0	2	10
0	0	11	12
0	0	8	13
2	0	5	14
1	0	9	11
0	0	10	11
2	0	19	2
5	2	2	16
0	0	4	17
4	0	3	12
1	0	23	0

[a] sup$^-$ bacteria of W3350 were infected with λb2susP3 at a multiplicity of 5 and incubated in broth at 37° for 180 min, and then infected with λb2susA11 at a low multiplicity of 0.01. Under these conditions, most of the cells which can produce phage particles upon induction were expected to be carrying one copy of each phage existing independently with each other. Single burst experiment was carried out just after the second phage infection in the same way as in Table 1. The contents of genotypes of each burst were described on each line of table. The recombination frequency was calculated to be 6.7 % by summing up these burst samples.

Table 3. Transduction of λb2 abortive prophage by phage P1. [a]

Time of P1 infection	Cell density at the time of P1 infection	Titer of infective P1 particles	Titer of λb2-transducing particles	λb2-transducing particles per initially infected λb2
60 min	1×10^8 cells/ml	2.0×10^7/ml		
150 min	7×10^7 cells/ml	1.9×10^9/ml	9.0×10^3/ml	9.0×10^{-5}
240 min	9×10^7 cells/ml	2.3×10^9/ml	8.5×10^2/ml	8.5×10^{-5}

a *sup*$^+$ bacteria of C600S were co-infected with λb2susA11 and λb2susP3 at a multiplicity of 5 for each, washed and resuspended in L-broth containing anti-λ serum at three different cell densities, namely 10^8, 10^7 and 10^6 cells/ml. The cultures were incubated at 37° for 60, 150 and 240 min, respectively, before the infection of P1, and continued to be incubated until lysis. Table shows that the titer of infective P1 and λ-transducing particles of these lysates. The 60 min's lysate gave a poor yield of P1 as well as the transducing particles.

Table 4. Single burst experiment of λ-transduced cells by phage P1. [a]

Genotype				No. of bursts
susA$^+$susP$^+$	susA11susP3	susA$^+$susP3	susA11susP$^+$	
0	0	12	0	12
0	0	0	12	19
7	0	5	0	12
9	0	3	0	
2	0	0	10	
3	0	0	9	
1	0	0	12	
9	0	0	3	
0	4	0	8	
0	5	0	7	
0	2	0	10	
0	3	0	9	
0	1	0	12	
0	1	12	0	
10	0	1	1	2
1	2	5	4	
12	0	0	0	2
12	0	0	0	

a Recipient cells of C600 irradiated by UV were infected with P1 prepared in Table 3 (150 min's sample) and highly diluted for single burst experiment. A dozen plaques from each burst were randomly picked and tested for their sus characters by complementation. The contents of genotypes in each burst were described on each line of table.

Table 5. P1 transduction of three different alleles of the immunity locus. [a]

Immunity markers	No. of plaques (%)
λc^+ λc 21	6 (1.6)
λc^+ λc	60 (16.1)
λc^+ 21	13 (3.4)
λc 21	57 (15.2)
λc^+	60 (16.0)
λc	109 (29.0)
21	70 (18.7)

a λb2, λc60 and λb2b5 were mixedly infected on C600S at a multiplicity of 5 for each and incubated in broth supplemented with anti-λ serum for 150 min at 37° before the infection of P1, and continued to be incubated until lysis. P1 lysate thus prepared was used for the transduction, which was made with C600S as the recipient and C600S/P1 as the indicator for λ plaques transduced. 375 of well isolated plaques were randomly picked and suspended in a small amout of buffer separately. The phage suspensions were spotted on the lawns of C600S(λ) and C600S(λb5) for testing their immunity characters. Table shows the immunity markers contained in a single plaque and the number of such plaques.

Table 6. Transduction of integrated or abortive monomer prophage of λ by small particles of phage P1. [a]

Recipient (UV-irradiated)	imm$^{\lambda}$-transductant imm$^{\lambda c}$	imm$^{\lambda c^+}$
W3350	0	20
W3350(λimm^{434})	259	1346 (h/h$^+$=2/98)
W3350(λimm^{434}h)	220 (h/h$^+$=18/82)	1245
W3350(λimm^{434}susR5)	190	884
W3350(λimm^{434}susA11)	43	357
W3350(λimm^{434}susA11susR5)	35	218

a W3623recA42(λh) was infected with λc60 at a multiplicity of 2. After 100 min's incubation, the culture was infected with P1 and led to be lysed. As the yield of P1 on recA strain was very poor, 50 ml of lysate was concentrated, followed by a sucrose density-gradient centrifugation to purify small particles and used for transduction of imm$^{\lambda}$ gene. Recipients used were W3350, W3350(λimm^{434}), W3350(λimm^{434}h), W3350(λimm^{434}susR5), W3350(λimm^{434}susA11) and W3350(λimm^{434}susA11susR5) after UV irradiation. Table shows the number of imm$^{\lambda}$-transductants for cI$^+$ and cI markers on the indicator of W3350(λimm^{434}-susA11susR5)/P1.

Table 7. Transduction of λb2 prophage in multiply infected cells by small particles of phage P1. [a]

Recipient (UV-irradiated)	imm$^{\lambda}$-transductant			
	imm$^{\lambda}$c h^{+}	imm$^{\lambda}$c h	imm$^{\lambda}$c^{+} h^{+}	imm$^{\lambda}$c^{+} h
W3350	2	0	0	0
W3350(λimm^{434})	283	32	136	136
W3350(λimm^{434}susR5)	20	5	107	143
W3350(λimm^{434}susA11)	235	21	26	19
W3350(λimm^{434}susA11susR5)	2	0	4	4

a The culture of C600S was mixedly infected with λb2c60susR5 and λb2hsusA11 at a multiplicity of 5 for each, washed and resuspended in L-broth containing anti-λ serum . The suspension was incubated with shaking at 37° for 120 min before the infection of P1, followed by further incubation until lysis. After the concentrating of P1 particles obtained, small particles were purified by a sucrose density-gradient centrifugation and used for transduction of imm$^{\lambda}$ gene. Recipients used were W3350, W3350(λimm^{434}), W3350(λimm^{434}susR5), W3350(λimm^{434}susA11) and W3350(λimm^{434}susA11susR5) after UV irradiation. Table shows the number of imm$^{\lambda}$ transductants for cI^{+} and cI markers on the indicator of W3350(λimm^{434}susA11susR5)/P1.

FIGURE LEGENDS

Fig. 1. Segregation of plaque-formable cells in a permissive strain infected with λb2cI857.

sup$^+$ bacteria of C600 were infected with λb2cI857 at a multiplicity of 10 (A) or 5 (B) and incubated in broth supplemented with anti-λ serum at 30°. (—●—) Number of colony formers; (—○—) Phage-producing cells after thermo-induction. Abscissa and ordinate show the time in hr after infection and the number of bacteria per ml, respectively.

Fig. 2. Segregation of plaque-formable cells in a non-permissive strain co-infected with two sus derivatives of λb2.

sup$^-$ bacteria of W3350 were co-infected with λb2susA11 and λb2susP3 at a multiplicity of 5 for each and incubated in broth at 37°. When the number of cells reached about 5×10^8 cells/ml, the culture was diluted ten-folds. The number of cells is expressed as the number calculated for the undiluted culture. (—●—) Number of colony formers; (—○—) Phage-producing cells after UV irradiation.

Fig. 3. Segregation of plaque-formable cells in a non-permissive strain co-infected with two sus derivatives of λb2tsP34.

sup$^-$ bacteria of 594 were co-infected with λb2susE4tsP34 and λb2susL63tsP34 at a multiplicity of 5 for each. After adsorption period of 20 min at 42°, the culture was diluted and divided into two portions. One was incubated at 30° (A) and the other at 42° (B). (—●—) Number of colony formers; (—○—) Phage-producing cells at 30° after UV irradiation.

Fig. 4. Segregation experiments at 30° using the various combinations of rec, red and int mutants. (—●—) Number of colony formers; (—○—)

Phage-producing cells after thermo-induction. (A) 594A co-infected with λcI857int2redB114susE4 and λcI857int2redB114susR5; (B) 594 co-infected with λcI857int2susE4 and λcI857int2susP3; (C) 594 co-infected with λcI857-int2redB114susE4 and λcI857int2redB114susR5; (D) 594A co-infected with λcI857int2susE4 and λcI857int2susP3; (E) 594A co-infected with λb2cI857-redB114susL63 and λb2cI857redB114susP3; and (F) 594A co-infected with λb2cI857redB114susL63 and λb2cI857redB114susE4.

Fig. 5. The model of complex genomes. (A) end-to-end cohesion; (B) cross over joining.

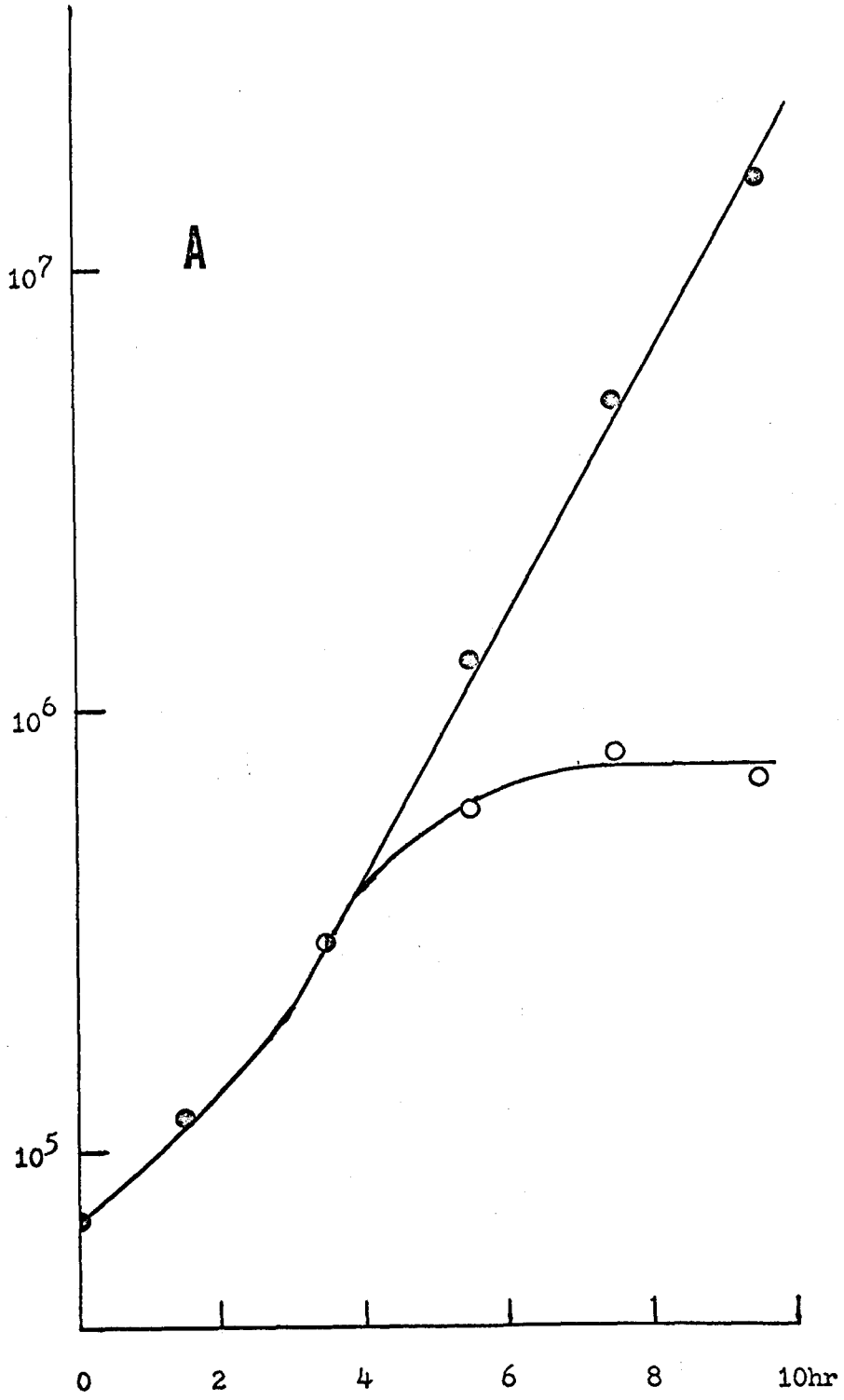


Fig 1 (+)

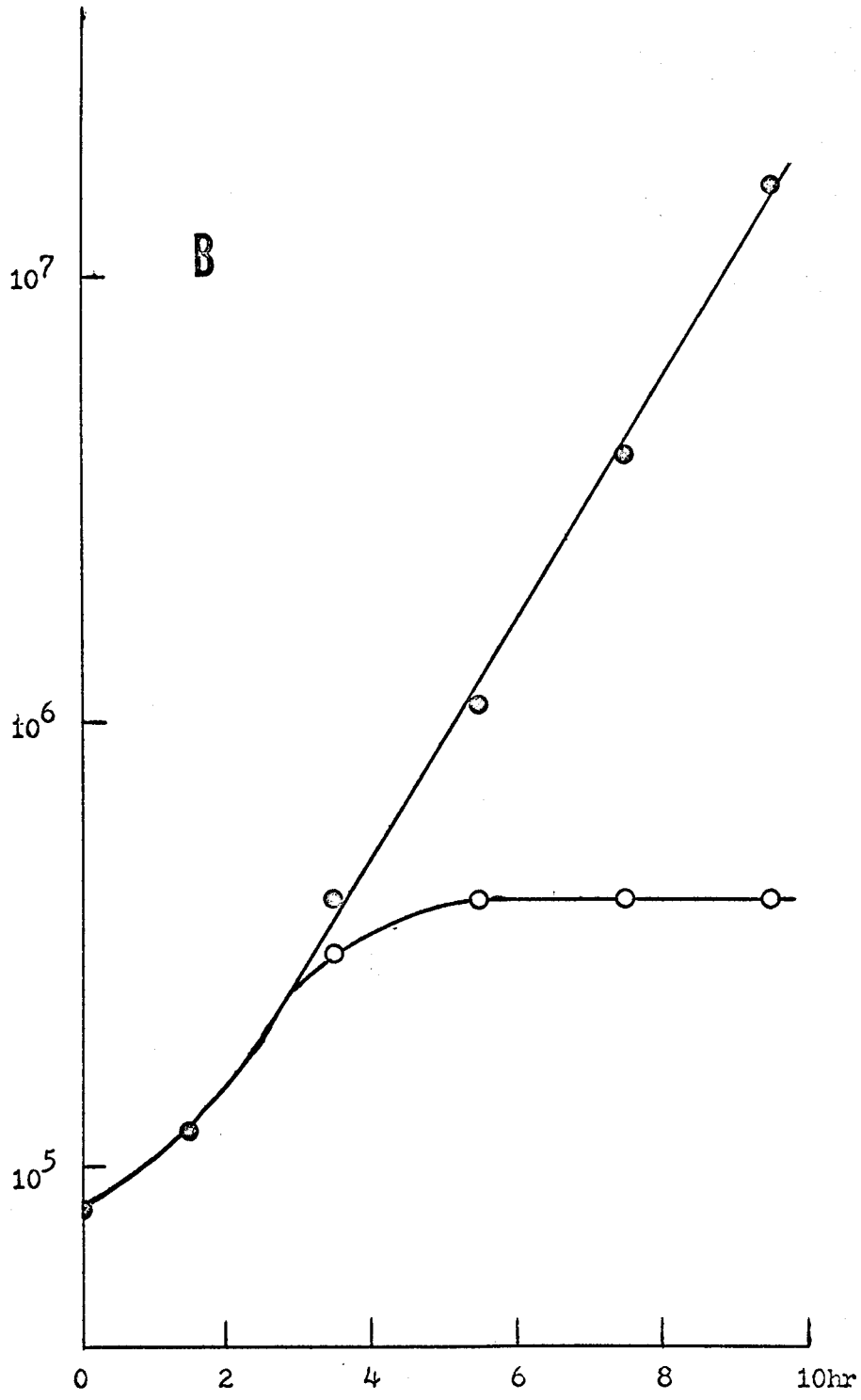
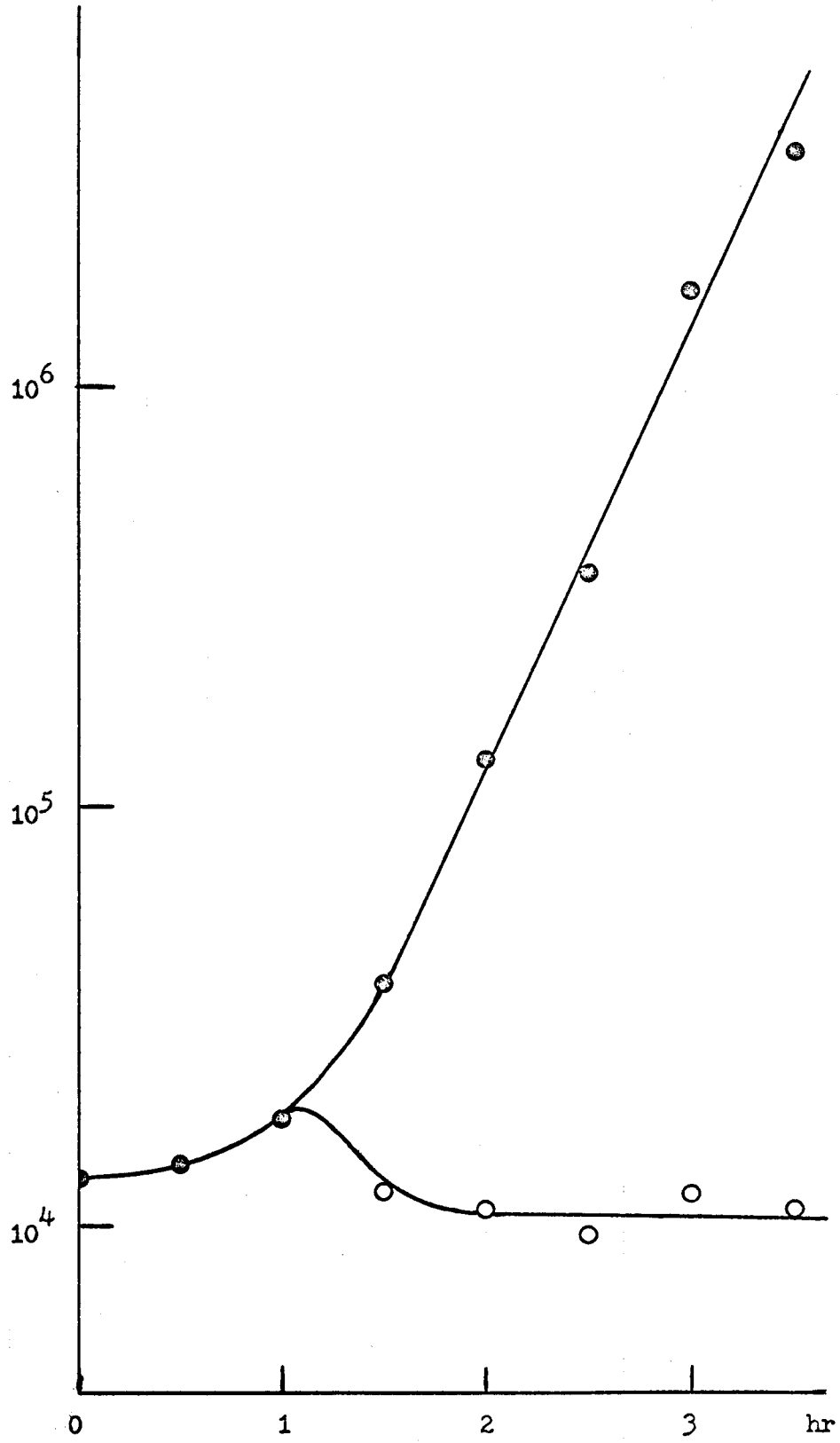


Fig 1 (B)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10^4
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1
2
3
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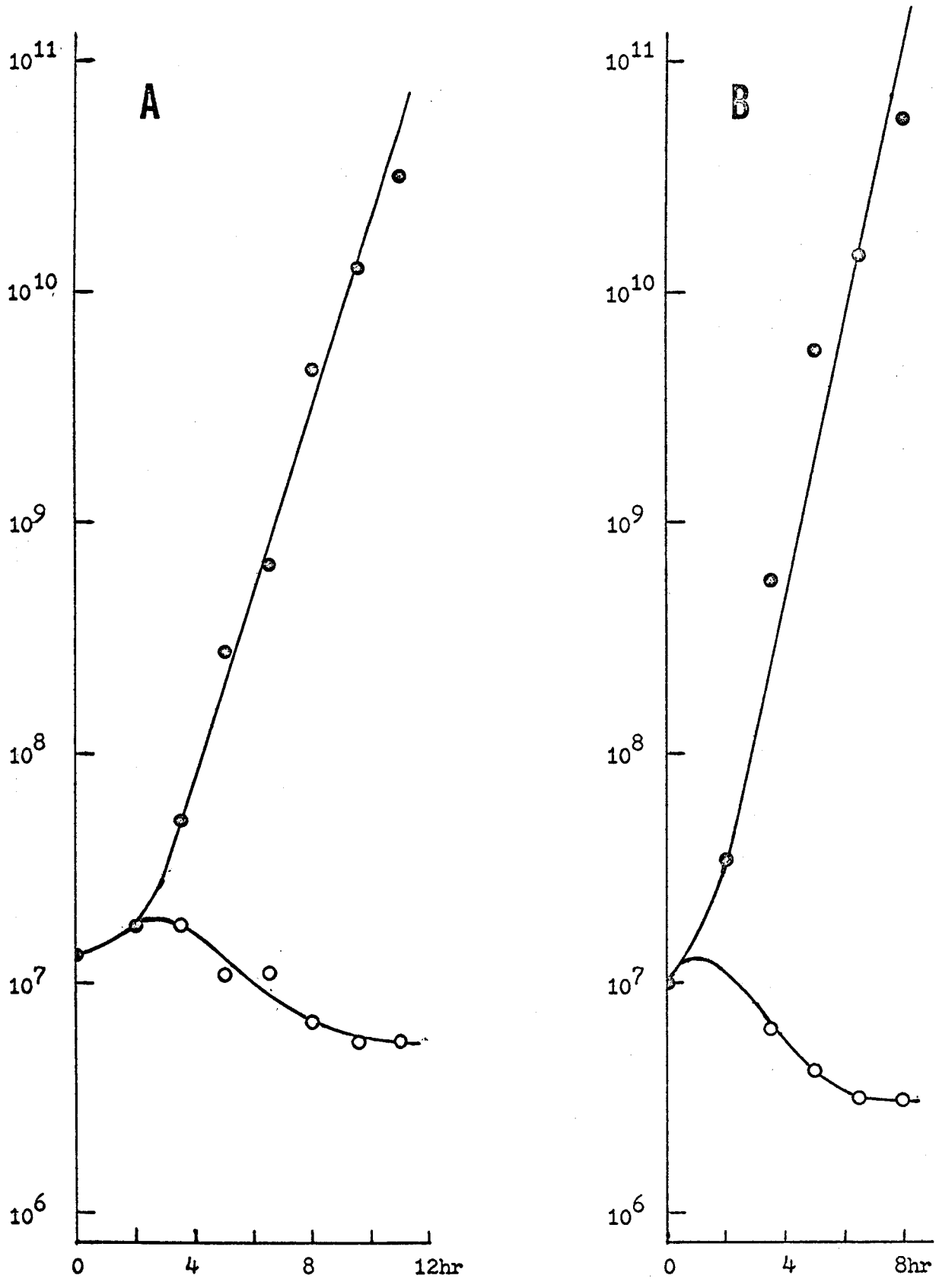
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B
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10^{8}
10^{7}
10^{6}
0
4
8
12hr
8hr

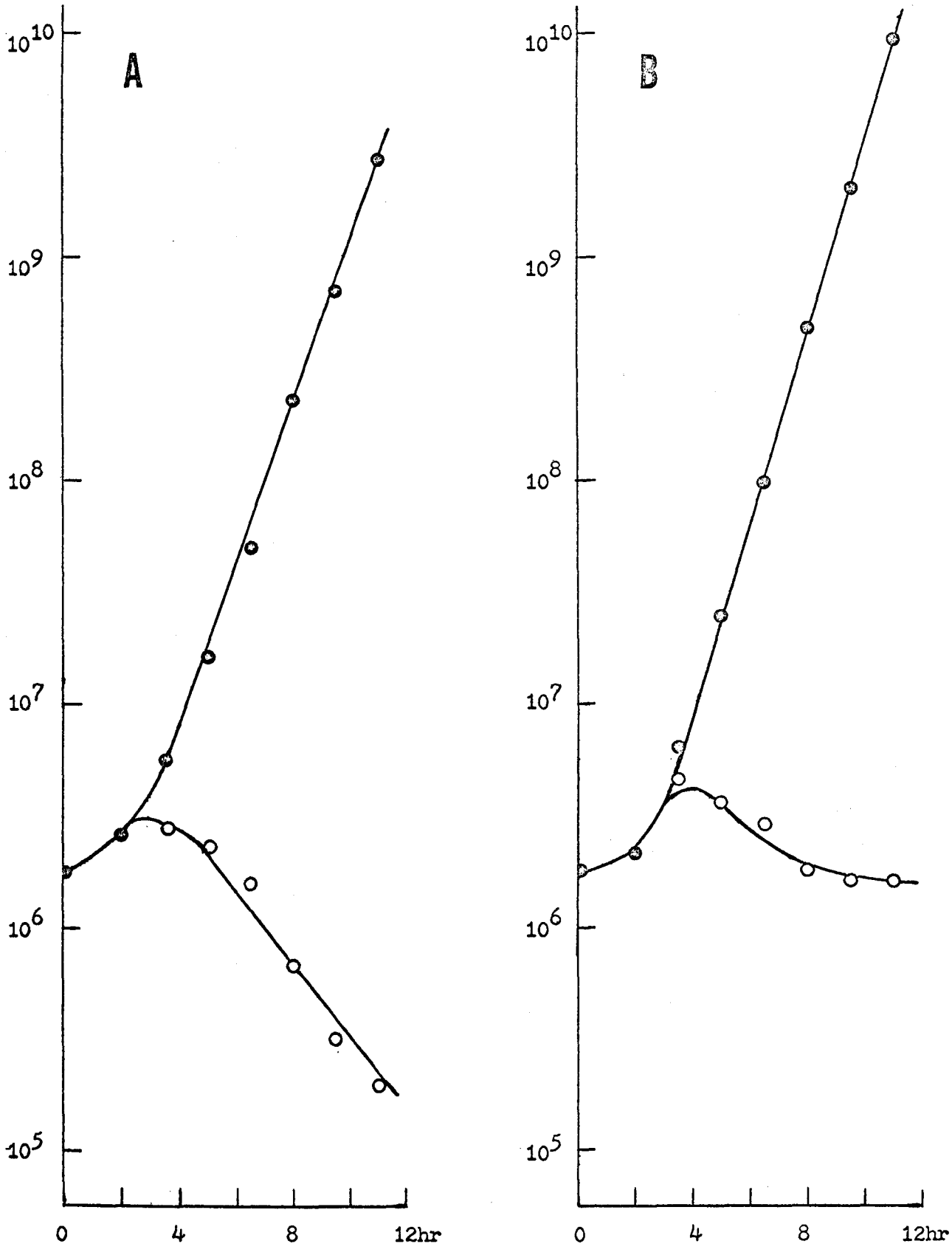


Fig 4 (A) (B)

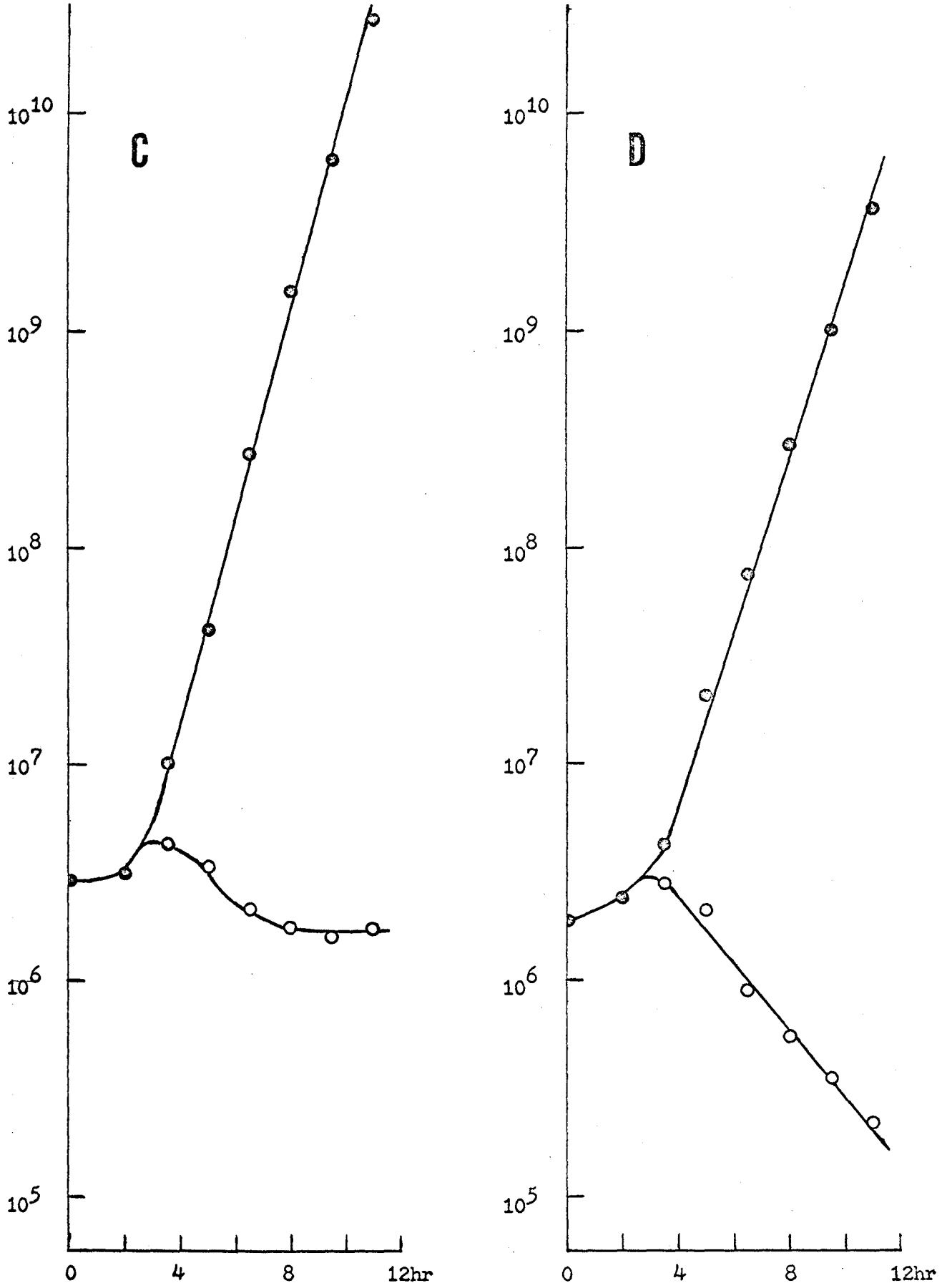
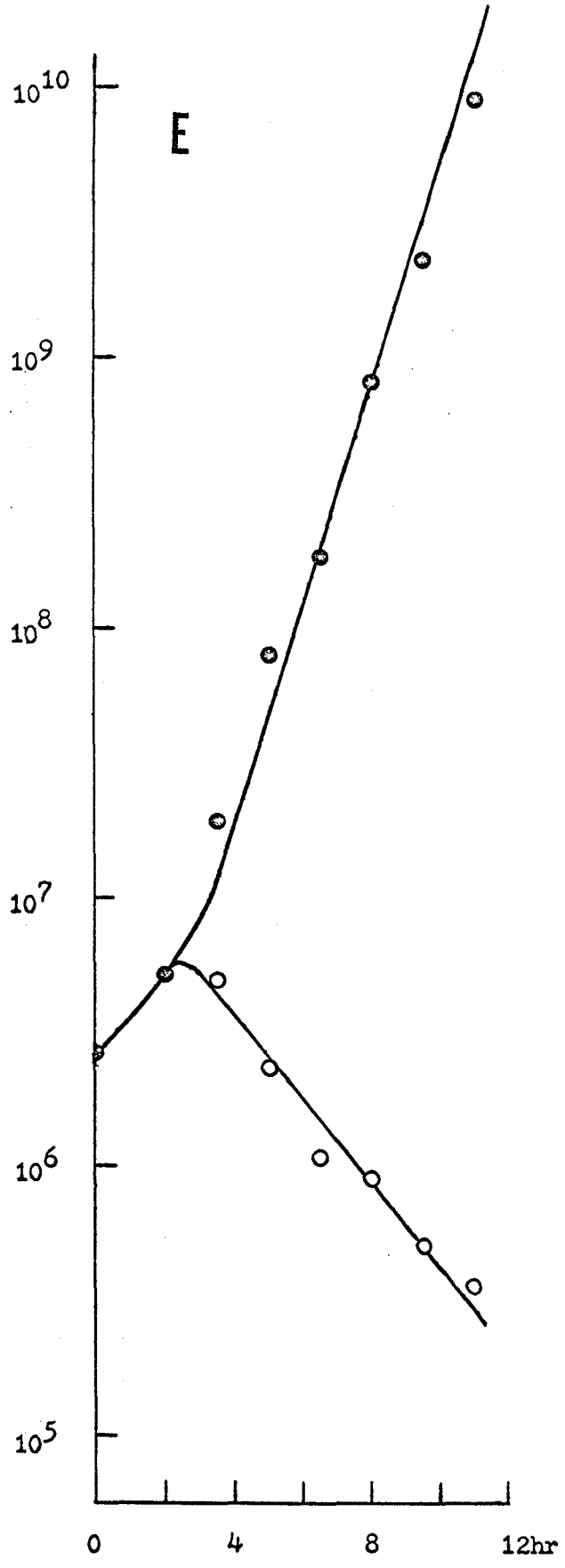


Fig. 4(1)(2)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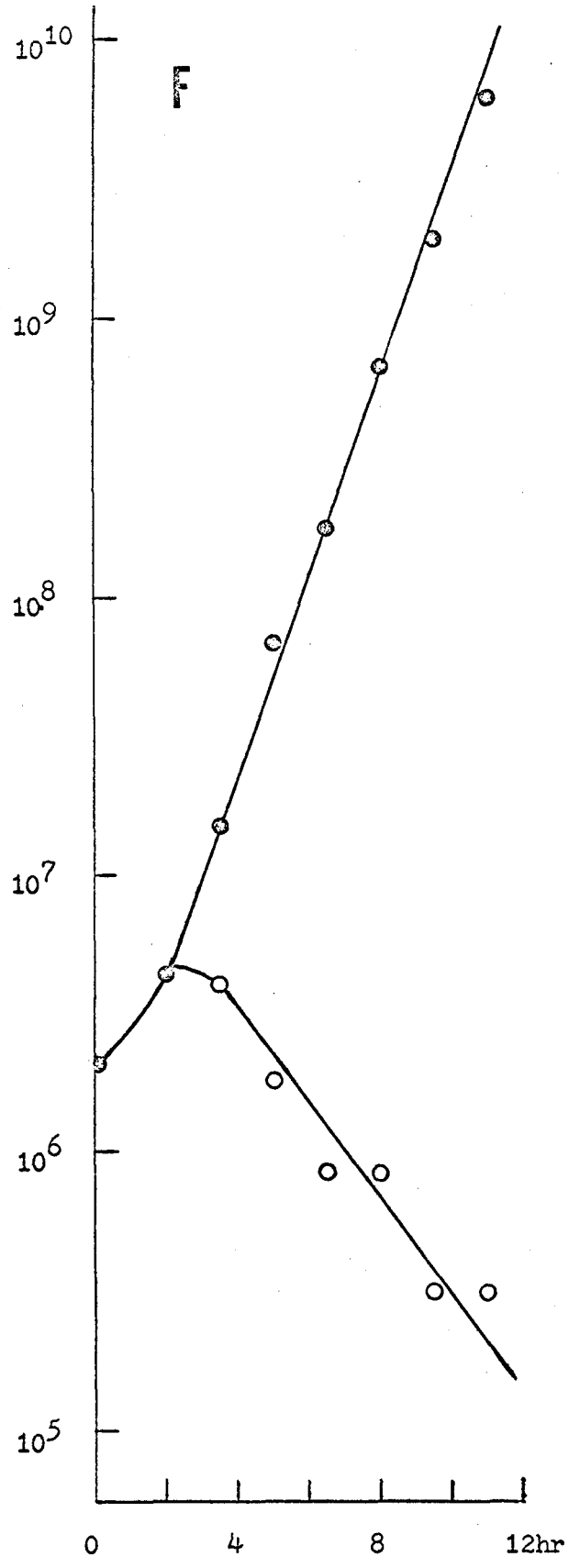
8
12hr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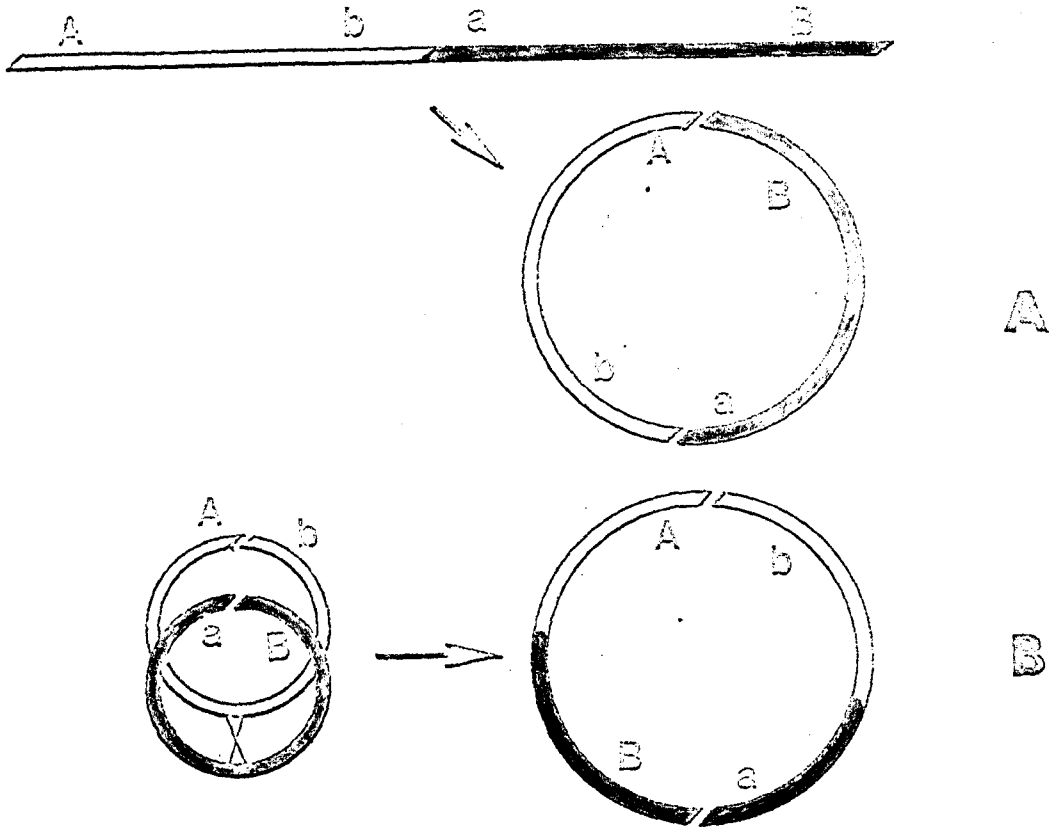
8
12hr

Fig 4 (a) (b)



A

B

2.8 5