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Dimeric Circular Duplex DNA of Bacteriophage ΦX174 and Recombination

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Summary. Bacteriophage ϕX174 replicative from DNA (RF DNA) was formed in the presence of chloramphenicol at a concentration of 40 μg per ml and isolated at 12 and at 55 min. after infection. The component I RF DNA (double stranded covalently closed and twisted form) was separated and divided into a monomer and multimer (dimer) fraction.

The frequency of recombinants found after phage formation in the chloramphenicol treated cells and that found after spheroplast infection with the monomer molecules both increase with the time of RF formation. However, the frequency of recombinant molecules among the dimers remained constant. This finding is explained by the hypothesis that two separate mechanisms act in ϕX174 recombination, one of which is restricted to the formation of dimers.

Irradiation with UV of phage prior to infection showed that the frequency of recombinants in monomers increased, as the recombination frequency of phage after (a single) growth (step) did, but that neither the frequency of recombinant molecules in dimers is raised, nor the frequency of dimers. Using a recombination negative host the frequency of recombinant dimer molecules was three to fourfold decreased, whereas the frequency of dimers was only slightly lower (relative to the normal host). These results support the hypothesis mentioned above and moreover lend support to the view that the greater part of the dimers is not formed by recombination events.

Introduction

Covalently closed circular duplex DNA molecules have been isolated from many sources. Among them two types of multiple circular DNA forms have been observed: circular oligomers, consisting of two or more genomes tandemly linked, and catenated oligomers consisting of closed circles that are interlocked.

These complex forms of circular DNA have been observed among bacterial plasmid DNA (Roth and Helinski, 1967; Rush *et al.*, 1969; Lee and Davidson, 1970), in cells infected with bacteriophages ϕX174 and S13 (Rush and Warner, 1967; Gordon *et al.*, 1970), M13 (Jaenisch *et al.*, 1969) and P22 (Rhoades and Thomas, 1968). Among mitochondrial DNA from different sources variable amounts of oligomers have been observed (Hudson and Vinograd, 1967; Clayton and Vinograd, 1967; Piko *et al.*, 1968; Nass, 1969). Circular and catenated oligomers have also been obtained from polyoma DNA (Cuzin *et al.*, 1970) and SV40 DNA (Jaenisch and Levine, 1971).

Two mechanisms have been proposed for the formation of multiple circular DNA molecules: recombination events (Hudson and Vinograd, 1967; Rush and

Warner, 1968a, b), and errors in DNA replication (Goebel and Helinski, 1968; Goebel, 1971).

In the present work the formation of dimers of circular duplex DNA of ϕX174 phage and recombination are compared.

Materials and Methods

1. Media

For phage growth and phage titrations the media described by Borrias *et al.* (1969), and for preparation, infection and plating of spheroplasts the media described by Guthrie and Sinsheimer (1963) were used.

2. Bacterial Strains

Escherichia coli C122 (BTCC no 122, abbreviated to *E. coli* C) is suppressor negative. *Escherichia coli* K58 (K58), kindly provided by Dr. Zinder (see Zinder and Copper, 1964), contains an amber suppressor and is ϕX174 resistant. *Shigella paradysenteriae* Y6R (Sh Y6R) carries an amber suppressor. *E. coli* HF4712, kindly provided by Dr. Sinsheimer, is a ϕX174 sensitive *recA* strain and contains an amber suppressor.

3. Phage Mutants

*Ts*27 is a temperature-sensitive mutant, unable to grow at 41°C, but showing normal growth at 30°C. Tourmaline 8 (*to*8) and emerald 7 (*em*7) are suppressor-sensitive (*sus*) mutants, able to grow on Sh Y6R and HF4712, but not on *E. coli* C. These mutants are described by Borrias *et al.* (1969).

4. Growth and Purification of Phage

Phages were grown in 5–1 batches and purified, as described by Borrias *et al.* (1969). For the UV-experiments the phage suspension after lysozyme treatment was centrifuged at 10000 rev. per min. in a MSE centrifuge. The supernatant was centrifuged in the No. 30 rotor of a Spinco model L ultracentrifuge at 30000 rev. per min. for 2 hr. The supernatant was discarded and the pellet was suspended overnight in 40 ml saturated borate – 0.025 M EDTA. Differential centrifugation at 10000 rev. per min. and 40000 rev. per min. was repeated. The final pellet was suspended in 10 ml borate-EDTA.

5. Preparation of RF-DNA

Replicating form DNA (RF-DNA) was, with a slight modification, prepared according to the method of Jansz *et al.* (1966). The procedure involved infection of *E. coli* C at 37°C (10^9 cells per ml.) with two ϕX174 mutants at a multiplicity of 5 for each phage, followed by the addition of chloramphenicol (CAP, 40 μg per ml.) at 9 min. after infection. The culture was vigourously aerated for 12 or 55 min. Cells were washed, and lysed with alkali at pH 12.3 and neutralized. The lysate was treated with RNase, heated to 80°C, and after removal of a precipate by centrifugation, concentrated by rotary flash evaporation to a final volume of 50 ml. This material was chromatographed on Sephadex G-100. The excluded fractions were passed on a nitrocellulose filter which retained the single stranded material. The eluate was concentrated by rotary flah evaporation to 10–20 ml.

Pure RF component I was prepared by ethidium bromide-CsCl equilibrium centrifugation by the procedure of Radloff *et al.* (1967). Ethidium bromide was added to a DNA solution to a final concentration of 150 μg per ml. CsCl was added (1 g per ml. of DNA solution in 0.001 M tris pH 7.5) and the refractive index was adjusted to 1.3845 by adding either CsCl crystals, or buffer. The solution was then centrifuged in a fixed-angle no 50 Spinco rotor (8 ml per tube and filled to the cap with paraffin oil) for 64 hr. at 38.000 rev. per min. at 10°. Samples were collected through a hole punctured in the bottom of the tube. The ethidium bromide was removed with dowex-50 resin (Na^+ form).

Irreversibly denatured RF component I was prepared by adding 0.75 N NaOH to RF component I (50–100 μg per ml. in 0.6 M NaCl-0.05 M tris) to a final pH of 12.8. After 10 min. at this high pH, the sample was neutralized with 1 N HCl to pH 7.5.

6. Biological Activity of ϕX174 DNA

Infective DNA was assayed in spheroplasts of K58. They were prepared according to Guthrie and Sinsheimer (1963). For the assay one volume DNA was mixed with one volume spheroplasts. After adsorption for 5 min. at 30°, 8 volumes PAM medium were added, and the suspension was shaken for 3 hr. at 30°C. The spheroplasts were lysed with chloroform.

7. Determination of Recombination Frequency of ϕX174 Phage

E. coli C was grown to a titer of 3×10^8 cells per ml. at 37°C. At 30°C the culture was infected with a mutant pair. 90 min. after infection cultures were shaken with chloroform and titered, usually on *E. coli* C and Sh Y6R. The recombination frequency was defined as the ratio of the titer on *E. coli* C at 41° (restrictive condition for *ts* and *sus* mutants) and the total yield (generally yield scored on *E. coli* C at 30°C plus yield on Sh Y6R at 41°C).

8. Determination of the Frequency of Recombinant DNA Molecules in a Spheroplast Assay

The frequency of wild type recombinant DNA molecules after DNA isolation from a mixedly infected culture was measured by infecting K58 spheroplasts at 30°C with the isolated DNA at a low multiplicity of infection (m.o.i.). Among the yield of the spheroplasts the frequency of wild type recombinants was scored as the ratio of wild type recombinants and the total yield.

9. Ultraviolet Irradiation

Ultraviolet irradiation was performed by illumination of phage or DNA samples with a low-pressure mercury tube (Philips, 30 W TUV).

10. Isolation of Dimers and Monomers from Alkali Denatured RF Component I by Sucrose Gradient Centrifugation

Irreversibly alkali denatured RF component I DNA was separated in a fast and slow sedimenting peak by repeated sucrose gradient centrifugation in two or three successive runs (Fig. 1). We will refer to the 38S peak as monomers and to the faster sedimenting material as dimers. The centrifugation was carried out by sedimentation through 4–20% linear sucrose gradients at 23000 rev. per min. in a SW25.1 or at 60000 rev. per min. in a SW65 rotor in a Spinco model L2 or L2–65 ultracentrifuge at 4°C. Samples were collected through a hole punctured in the bottom of the tube. Alkali denatured RF component I was used instead of native RF component I, because of the greater difference between the S values of monomers and dimers as compared to those of native RF component I.

11. Electron Microscopy

Samples for the electron microscope were prepared according to the method of Kleinschmidt and Zahn (1959). The spreading solution contained 1–2 μg DNA per ml. in 0.25 M ammonium acetate and 0.02% cytochrome c. Before spreading, the DNA sample in 0.6 M NaCl–0.1 M phosphate was heated for 1 hr. at 85°C. The subphase consisted of 100 ml. 0.25 M ammonium acetate in a petri-dish coated with parafilm. Mica sheats were used to apply the spreading solution. The monolayer was transferred onto 400 mesh grids coated with carbon film. The grids were washed with aquabidest and dried with ethanol. The film covered grids were rotary shadowed with platinum at an angle of 5–7°. Electron micrographs were taken with a Philips EM 200 electron microscope at magnifications of 5000–10000. For measurement of the contour lengths of the circular DNA, the covalently closed circular DNA was exposed to 85°C in 0.6 M NaCl–0.1 M K. phosphate pH 6.8 for one hour (Tieze *et al.*, 1969).

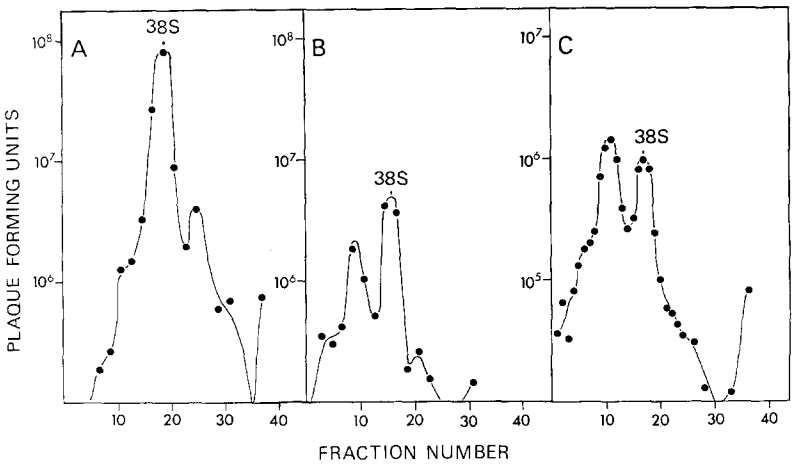

Fig. 1 A—C. Isolation of dimers by repeated sucrose gradient centrifugation. A 100 μg alkali denatured component I DNA was layered on top of a 27 ml. linear 4–20% sucrose gradient in 1M NaCl–0.005 M Tris–0.025 M EDTA pH 7.5. After 10 hr. centrifugation in a SW 25[I] rotor of the Spinco L_2^{65} at 23000 rev. per min. and at 4°, fractions were collected. The fractions 4–15 from two gradients according A were pooled, dialyzed and concentrated. B The pooled material from A was divided over two 5 ml linear 4–20% sucrose gradients and centrifuged during 90 min. in a SW65 rotor of a Spinco L_2^{65} at 60000 rev. per min. at 4°. Fraction 3–12 were pooled, dialyzed and concentrated. C The combined fractions 3–12 from B were layered on top of a 5 ml. linear 4–20% sucrose gradient and centrifuged at 60000 rev. per min. and 4° during 90 min. The biological activity of the various fractions has been determined. For that purpose samples of the fractions of A and B were diluted 1:100 and of C 1:50 in 0.01 M HMP buffer. With these samples a sferoplast infection was performed in sferoplast of K58 at 30°

Results

1. Frequency of Recombinant Molecules among Monomers and Dimers, Isolated at 12 and 55 Minutes after Infection

Rush and Warner (1968a, b) found that the frequency of recombinant molecules among RF component I, isolated 60 min. after infection, is equal to the recombination frequency measured in a normal phage cross. We found however, that the frequency of recombinant molecules among RF component I from a 55 min.—RF-culture was about three times higher than the recombination frequency in the corresponding phage cross. In fact the latter result is what one can expect, on the basis of the following experiments.

Firstly the frequency of ϕX174 recombination after spontaneous lysis is enhanced by increasing the period of RF formation in the presence of CAP, at 40 μg per ml. (Fig. 2). There is an almost linear relationship between recombination frequency and time of sampling. The increase after 55 min. is a factor of 3 to 4 compared to the recombination frequency without CAP. Secondly a similar linear relationship was obtained when the frequency of recombinant molecules was determined among parental RF and RF component I isolated at 12 and 55 min. after infection. Parental RF and RF at 12 and 55 min. after infection was obtained from *E. coli* C, infected with the mutant pair *to*8 × *ts*27, as indicated in the legend

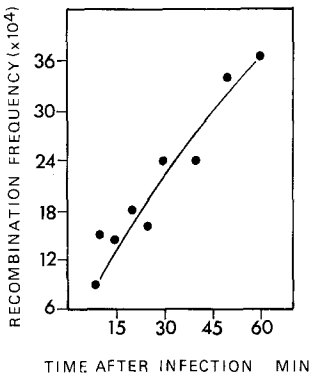

Fig. 2. Increase in recombination frequency during growth in the presence of 40 μg CAP per ml. *E. coli* C grown to a concentration of $4 \cdot 10^8$ cells per ml. in 3×D medium at 37°, was mixedly infected with the mutant pair *ts*27×*to*8 (m.o.i. 5+5). RF replication was allowed to proceed in the absence of the formation of single stranded DNA and of progeny phage by the addition of CAP (40 μg per ml) at 9 min. after infection (Sinsheimer *et al.*, 1962). Samples were taken at various periods after infection, diluted into fresh medium without CAP at 30° and aerated vigourously. After spontaneous lysis the frequency of wild type recombinants in the lysate was determined

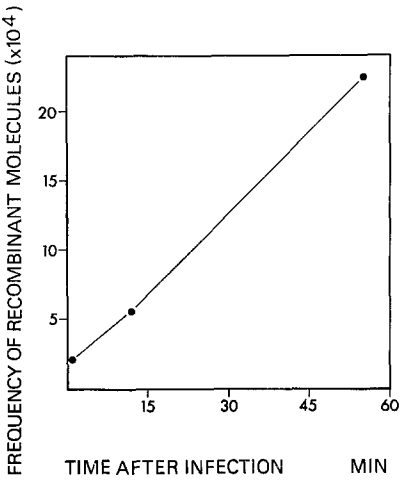

Fig. 3. Frequency of recombinant molecules in RF component I, isolated at different times after infection. *E. coli* C, grown to a concentration of $4 \cdot 10^8$ cells per ml in 3×D medium, was mixedly infected with the mutant pair *ts*27×*to*8. CAP was added to a final concentration of 40 μg per ml. at 9 min. after infection. From a part of the culture RF component I was isolated 12 min. after infection and from the rest at 55 min. after infection. For point zero CAP was added to a final concentration of 150 μg per ml at ten min. before infection. In the latter case RF component I DNA was isolated 12 min. after infection. The frequency of recombinant molecules among RF component I was determined as described in materials and methods

to Fig. 3. During progeny RF synthesis, we observe an increase in the frequency of recombinant molecules (Fig. 3).

The increase of the recombination frequency and the frequency of recombinant molecules might be due to the longer period of RF formation, to the larger DNA

pool which accumulates under these conditions, or to a direct effect of CAP on recombination.

Rush and Warner (1967) separated RF component I in monomers and dimers. Rush and Warner (1968a, b) hypothesized that dimers are intermediates in the recombination process of the bacteriophages ϕX174 and S13 on account of the finding, that the frequency of recombinant molecules among dimers was 10 to 15 times higher than among monomers from the same 60 min.-RF-culture. In agreement with Rush and Warner we found that the frequency of recombinant molecules among dimers is higher than among monomers. In our experiments however the dimer fractions contained only twice as many recombinant molecules as the monomer fractions for the mutant pairs *to*8 × *ts*27 and *em*7 × *ts*27, when the RF component I DNA was isolated at 55 min. after infection (Table 1).

Table 1. Frequency of recombinant molecules

Host	Mutants	Time after infection the RF culture was stopped (min.)	Frequency of recombinant molecules among			Recombination frequency after spontaneous lysis
			Component I DNA	Monomers	Dimers	
E. coli C	*to*8 × *ts*27	12	8×10^{-4}	7×10^{-4}	57×10^{-4}	8×10^{-4}
E. coli C	*to*8 × *ts*27[a]	12	16×10^{-4}			34×10^{-4}
E. coli C	*to*8 × *ts*27	55	28×10^{-4}	28×10^{-4}	51×10^{-4}	27×10^{-4}
E. coli C	*to*8 × *ts*27[a]	55	38×10^{-4}	36×10^{-4}	60×10^{-4}	64×10^{-4}
E. coli C	em7 × *ts*27	55		56×10^{-4}	122×10^{-4}	
HF4712	*to*8 × *ts*27	55		3×10^{-4}	15×10^{-4}	2×10^{-4}

RF DNA was prepared as described in Materials and Methods. Immediately after stopping the culture, a sample was diluted 10^4 in fresh medium and vigorously aerated to score the recombination frequency after spontaneous lysis. Component I DNA was isolated and separated in monomers and dimers. The frequency of wild type recombinant molecules was determined for the total component I DNA and isolated monomers and dimers as described in Materials and Methods.

[a] Before infection the phages were UV irradiated to a survival of 22%.

According to the hypothesis of Rush and Warner (1968a, b) one expects at 12 and 55 min. after infection a constant and high frequency of recombinant molecules among dimers and an increase in the frequency of recombinant molecules among the monomers from 12 to 55 min. as a consequence of the segregation of heterozygous dimers to recombinant monomers. To verify this, we isolated RF component I DNA at 12 and 55 min. after infection. The frequencies of recombinant molecules among dimers at 12 and 55 min. after infection are equal. At 12 min. after infection the frequency of recombinant molecules among dimers is sevenfold higher than among monomers and at 55 min. twofold (Table 1). These results seem to support the hypothesis that dimers are intermediates in recombination and that recombinant monomers are formed by segregation of heterozygous dimers.

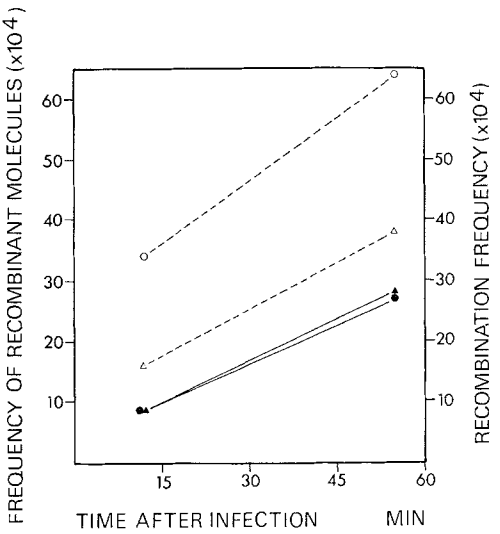

Fig. 4. Recombination frequency and frequency of recombinant molecules with and without UV irradiation of the phages before infection. Cultures of *E. coli* C in 3×D medium, grown to a final concentration of $9 \cdot 10^8$ cells per ml were mixedly infected with the mutant pair *ts*27 × *to*8 (m.o.i. 5+5) 9 min., after infection CAP was added to a final concentration of 40 μg per ml. At 12 and 55 min. after infection RF DNA was isolated. Before harvest of the cells a sample was diluted 10^4 in fresh medium, aerated vigourously and after spontaneous lysis the frequency of wild type recombinant molecules was determined. The percentage of dimers in total RF DNA was determined. After isolation of RF component I DNA the frequency of recombinant molecules was determined. Recombination frequency after spontaneous lysis: •——• after infection with unirradiated phages. ○——○ after infection with phages UV irradiated to a survival of 22%. Frequency of recombinant molecules among RF component I: ▲——▲ after infection with unirradiated phages. △——△ after infection with phages UV irradiated to a survival of 22%

From these data it cannot be ruled out, however, that recombinant monomers are formed by another mechanism. Recombinant dimers might be the end product of some kind of recombination event, and do not segregate to recombinant monomers. We have further tested these possibilities as follows.

2. Effect of UV Irradiation of the Phages on Recombination Frequency and Percentage of Dimers

UV irradiation of the bacteriophages S13 and ϕX174 prior to infection results in an increase in recombination frequency (Tessman and Shleser, 1963; Tessman, 1968). If there are two separate mechanisms for the formation of recombinant molecules, among monomers and among dimers respectively, UV irradiation of the phages prior to infection might stimulate one of these mechanisms only. We already saw, that the recombination frequency can be enhanced by increasing the period of RF formation in the presence of CAP at 40 μg per ml. (Fig. 2). The recombination frequency was further enhanced when we did the same experiment with UV irradiated phages (Fig. 4). We isolated RF component I DNA at 12 and

55 min. after infection from *E. coli* C infected with UV irradiated phages. The data (Fig. 4 and Table 1) of the 12 min. experiments indicate that UV irradiation of the phages prior to infection, results in a 4 fold increase in the recombination frequency of the phages after spontaneous lysis and in a 2 fold increase in the frequency of recombinant molecules among RF component I. For the 55 min. experiments a two to threefold increase in the recombination frequency of phages was found. The frequency of recombinant molecules among RF component I was increased with the same amount as after 12 min. (Fig. 4). However, there was no effect of UV irradiation neither on the frequency of recombinant molecules among dimers at 12 and 55 min. after infection (Table 1), nor on the percentage (about 4%) of dimers as determined by electron microscopy.

We can conclude from these data, that UV irradiation of the phages prior to infection stimulates a recombination mechanism, generating recombinant monomers and recombinant phages. It is reasonable to assume that dimers play no part in this stimulation. However, the possibility is still open that dimers are formed by some kind of recombination process, and do not segregate to recombinant monomers.

3. Percentage of Dimers and Frequency of Recombinant Molecules in RF Component I Obtained from a φX174 Infected RecA Host

The finding of the constant and high frequency of recombinant molecules among dimers supports the possibility that dimers are formed by some kind of a recombination event. The frequency, however, is not high enough to exclude the possibility that only a part of the dimers is formed by a recombination event and another part as a by-product of the normal replication process (Rush and Warner, 1968b). If a part of the dimers is formed by a recombination event, one may expect, that in a host deficient for ϕX recombination, the frequency of recombinant molecules among dimers is decreased, while the total percentage of dimers remains about the same.

Using strain HF4712 (*recA*) as a host for normal phage crosses ϕX recombination is decreased to about 10 percent of the normal value obtained for a rec$^+$ host. We determined the frequency of recombinant molecules among monomers and dimers of RF component I DNA, isolated from HF4712, mixedly infected with the mutant pair *to*8 $\times$ *ts*27. The frequency of recombinant dimer molecules was three to four times decreased compared to the frequency obtained for dimers isolated from ϕX infected rec$^+$ cells under the same conditions (Table 1).

By electron microscopy we determined the percentage of dimers among RF DNA molecules, isolated at 55 min. after infection of the ϕX174 infected hosts HF4712 (*recA*) and *E. coli* C (rec$^+$). We found 4% dimers for RF DNA isolated from HF4712 and 4.8% for RF DNA from *E. coli* C (in both cases 1000 molecules were scored). For RF component I DNA isolated from HF4712 and also for RF component I DNA from *E. coli* C we found 18% catenated and 82% circular dimers among dimers of the dimer peaks, obtained after repeated sucrose gradient centrifugation (in each case 300 dimer molecules were scored). In Fig. 5a monomer, a catenated dimer and a circular dimer are shown. So we could not correlate the recombination deficiency of the host and the percentage of dimers, either catenated or circular.

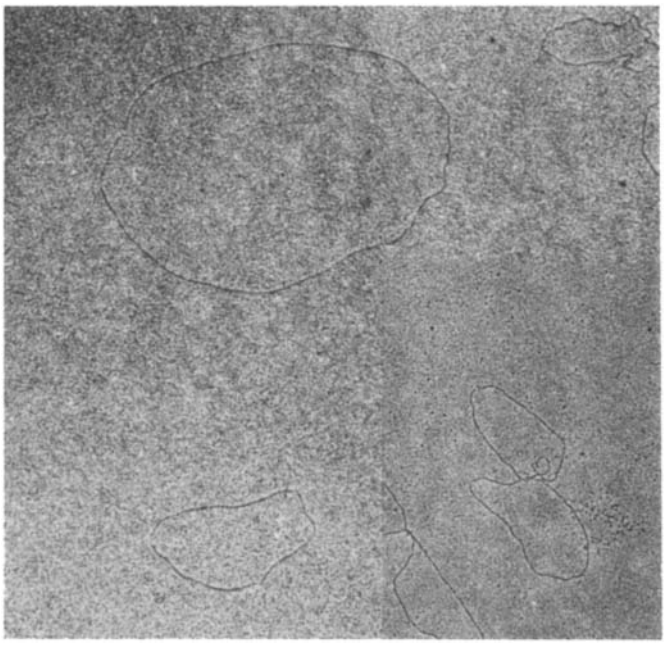

Fig. 5. Electron micrographs showing a monomer, a circular and a catenated dimer

The data that the frequencies of dimers among RF DNA from ϕX infected *recA* and *rec*$^+$ hosts are about the same, while the frequency of recombinant molecules among dimers in the *recA* host is decreased compared to the *rec*$^+$ host support the hypothesis that the major part of the dimers is not involved in a recombination event.

The frequencies of recombinant molecules among monomers and dimers isolated from the ϕX infected *recA* host are respectively ten and three times decreased compared to the frequency obtained for monomers and dimers isolated from ϕX infected *rec*$^+$ cells under the same conditions (Table 1). So the *recA* mutation has a stronger effect on the generation of recombinant molecules among monomers than among dimers. This is another indication that recombinant monomers and recombinant dimers are formed by two separate pathways.

Discussion

Several mechanisms have been proposed for the formation of multiple circular DNA molecules. They are either based on recombination events (Hudson and Vinograd, 1967; Rush and Warner, 1968a, b) or on errors in replication (Goebel and Helinski, 1968; Goebel, 1971). The results of Goebel (1971) with regard to the formation of oligomeric col E1 DNA in the temperature sensitive replication mutant *E. coli. ts* CR34/43 and two different recombination-deficient mutants carrying the same *ts* replication marker, support a replication-type model for the generation of circular and catenated multimers. The results of Rush and Warner

(1968a, b) seem to support a recombination-type model. Rush and Warner mixedly infected *E. coli* C with two temperature sensitive mutants of bacteriophage S13. They isolated monomeric and dimeric RF component I DNA 60 min. after infection. They scored a higher percentage of wild type recombinant molecules among dimers than among monomers. They hypothesized that dimers arise as a recombination product and are intermediates in the recombination process of ϕX174 and S13. Our finding of a high and rather constant frequency in time of recombinant molecules among dimers and an increased frequency in time among monomers, for RF component I DNA isolated from a ϕX infected *rec*$^+$ host at different times after infection, seems to support this hypothesis.

However, these results with S13 and ϕX174 cannot rule out the possibility that recombinant monomers and dimers are formed by two separate mechanisms. Inhibition of phage formation by 40 μg CAP per ml. results in the increase of recombinant monomers but not of recombinant dimers. Dimers might be the end product of some kind of recombination event, and thus will not segregate to recombinant monomers. From the UV experiments we obtained further support for the possibility that recombinant monomers and recombinant dimers are formed by two separate mechanisms. UV irradiation of the phages prior to infection stimulated the formation of recombinant molecules among RF component I monomers and the formation of recombinant phages. However, there was no effect of UV irradiation, either on the frequency of recombinant molecules among dimers at 12 and 55 min. after infection, nor on the percentage of dimers. So UV irradiation only stimulates the pathway, generating recombinant monomers and recombinant phages. It seems unlikely that dimers are intermediates for the generation of these recombinants, but dimers themselves may still be formed as an endproduct of a recombination event. The finding that the *recA* mutation has a stronger effect on the generation of recombinant monomers than on the generation of recombinant dimers also support the hypothesis that recombinant monomers and dimers are formed by two separate pathways.

According to their hypothesis Rush and Warner (1968b) expected to find 9.4% wild type recombinant molecules among dimers. Their experimental value, however, was 20 times lower. A possible explanation for the discrepancy between the expected and the experimental value is that ϕX multimers arise in two ways: as a by-product of the normal replication process and as a product of a recombination event. Our experiments with the *recA* host indicate that there are two mechanisms generating dimers. The frequency of recombinant molecules among dimers, isolated from the ϕX infected *recA* host was 3 to 4 times lower than among dimers from a ϕX infected *rec*$^+$ host under the same conditions, indicating that the *rec* character of the host influences the frequency of recombinant molecules among dimers. From electron microscopic examination we learned that there was only a small decrease in the total percentage of dimers. It seems likely that in the *recA* host the pathway that generates dimers by recombination is affected, but not another pathway, generating dimers probably as errors in replication. We conclude that dimers can arise by two mechanisms, one of which is working via a recombination event. Dimers do not segregate to recombinant monomers. Recombinant monomers are formed by a separate pathway, for example by the process proposed by Weisbeek and Van de Pol (1970).

Baker *et al.* (1971) proposed the existence of a primary and a secondary mechanism for the genetic recombination. In their opinion the primary mechanism should quantitatively be the major one, in which parental DNA is primarily involved. They could not ascertain, however, that the primary mechanism acts exclusively on parental DNA. We measured the frequency of recombinant molecules in the presence and in the absence of progeny DNA synthesis (Fig. 3) and found a considerable increase in this frequency under conditions in which besides parental RF a large amount of progeny RF is formed. From this observation we conclude that parental RF is not exclusively used as substrate for recombination, but on the contrary, that progeny RF is the main substrate.

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