J. Mol. Biol. (1972) **63**, 557–568

Asymmetric Information Transfer during ϕX174 DNA Replication

P. D. Baas and H. S. Jansz

Laboratory of Physiological Chemistry
Vondellaan 24a, State University
Utrecht, The Netherlands

(*Received 13 July 1971, and in revised form 2 November 1971*)

By annealing the single-stranded DNA of ϕX174 phage with the complementary strand obtained from the replicative form of this phage, heteroduplex RF† was prepared with a genetic marker in the viral strand, which is different from that in the complementary strand.

Heteroduplex RF was indistinguishable from native RF in buoyant density in CsCl, in contour length in the electron microscope and in hydroxylamine sensitivity. Heteroduplex RF is infectious to spheroplasts of the appropriate bacterial host. Infected spheroplasts yield a burst of approximately equal quantities of phage of the plus- and minus-strand type and the majority of the spheroplasts produces a single type of phage. This and other evidence indicates that the heteroduplex region is repaired before replication.

The ultraviolet sensitivity of heteroduplex RF DNA is intermediate between that of single- and double-stranded ϕX DNA suggesting that repair of a heteroduplex region involves the degradation of a large single-strand segment.

In a small fraction of the infected spheroplasts, heteroduplex RF escapes the repair process by replication, and these spheroplasts produce phage of both genotypes. The size of this fraction of mixed bursts depends on the position of the heteroduplex region on the genetic map of ϕX. Treatment of spheroplasts with mitomycin C inhibits heteroduplex repair. From the genotype of the progeny phage that is produced in mixed bursts it is concluded that the complementary strand of ϕX heteroduplex RF largely governs the RF replication.

1. Introduction

The replication cycle of the single-stranded DNA phage ϕX174 has been investigated intensively during the past decade largely by Sinsheimer and co-workers (for a review see Sinsheimer, 1968). After infection of the bacterial host, *Escherichia coli* C, the circular and single-stranded DNA of the phage is converted to a circular DNA duplex (replicative form), which then replicates to form a pool of RF†. The DNA of the progeny phage is derived from this pool; the phage strand is packaged into coat protein, whereas the remaining complementary strand serves as a template for the further synthesis of new phage strands (Lindqvist & Sinsheimer, 1968; Knippers, Komano & Sinsheimer, 1968; Komano, Knippers & Sinsheimer, 1968).

The present work is an attempt to obtain information on the mechanism of RF DNA replication. The experimental approach is as follows. Circular heteroduplex RF was constructed in which the phage strand (plus-strand) contains a genetic marker

† Abbreviation used: RF, replicative form.

which is different from that of the complementary strand (minus-strand). After infection of the appropriate bacterial host with heteroduplex RF, the progeny phage was analysed with respect to the genetic marker it contained. This analysis should decide whether the plus-strand, minus-strand or both strands transmit genetic information to the progeny phage, which in turn would yield information on the mechanism of replication of the RF of ϕX phage.

However, the results indicate that, in a large fraction of the infected spheroplasts, heteroduplex RF is converted to homoduplex RF before replication, presumably by a repair mechanism comparable to that acting on pyrimidine dimers in u.v.-irradiated double-stranded DNA (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964; Pettijohn & Hanawalt, 1964). In a small fraction of the infected spheroplasts heteroduplex RF escapes repair by replication. The size of this latter fraction appears to be a function of the position of the heteroduplex region on the genetic map of ϕX phage in the sense that there is a gradient from a fixed point along the genetic map in one direction. These experiments which allow the determination of the origin and direction of replication of ϕX RF will be described in the accompanying paper (Baas & Jansz, 1972). The present paper deals with an analysis of the fraction of spheroplasts in which heteroduplex RF undergoes replication. This fraction of spheroplasts is considerably increased by mitomycin C treatment, suggesting that this antibiotic specifically inhibits repair and not replication. From the genotype of the progeny phage that is produced in these spheroplasts, it is concluded that the minus strand of ϕX hetero–duplex RF governs the RF replication. A preliminary account of part of this work has already appeared (Baas & Jansz, 1971).

2. Materials and Methods

(a) *Bacterial strains* (E. coli)

Strain C (C0), the usual host of ϕX, was obtained from Dr R. L. Sinsheimer.
C1 and CH are resistant to the wild-type ϕX.
K12 is a ϕX-resistant strain used as the normal host in the spheroplasts assay for ϕX DNA. K12 *hcr*$^-$ is a mutant which has lost the property of host-cell reactivation (isolated by Harm).
K58 (*su*$^+$) is a ϕX-resistant strain used in the spheroplast assay for ϕX DNA.
H502 (*su*$^-$, *hcr*$^-$, *thy*$^-$ and Endo I$^-$) was isolated by Hofmann-Berling.
K12 RecA13 and RecB21 were isolated by Howard-Flanders & Theriot.
K12 RecC22 was isolated by Willetts & Mount.
W3110 *polA1*$^-$ was isolated by De Lucia & Cairns.

All bacterial strains grow in modified 3XD broth. This medium contains: 0·9 g KH_2PO_4, 2·1 g Na_2HPO_4, 1·0 g NH_4Cl, 1·0 ml. 1% gelatin solution, 15·0 g Casamino acids, 30·0 g glycerol, all made up to 1 l. and autoclaved. After sterilization 10 ml. $MgSO_4$ (6·1 g $MgSO_4 \cdot 7H_2O$/100 ml.) and 0·3 ml. 1 M-$CaCl_2$ are added. If necessary thymidine is added also to a final concentration of 2 μg/ml.

(b) *ϕX174 strains*

ϕ_0 is the wild-type ϕX phage obtained from Dr R. L. Sinsheimer.
ϕ_1 is a first-step host range mutant of ϕX (Jansz, Van Rotterdam & Cohen, 1966).
E. coli C1 is sensitive to ϕ_1.
A distinction between ϕ_0 and ϕ_1 phage can be made by plating on the mixed indicator *E. coli* C0 and *E. coli* C1. ϕ_0 phage produces a turbid plaque and ϕ_1 phage a clear plaque.
ϕ_6 is a first-step host-range mutant of ϕX. *E. coli* CH is sensitive to ϕ_6. For a distinction between ϕ_0 and ϕ_6 phage, the mixed indicator *E. coli* C0 and *E. coli* CH is used.
On account of the percentage of mixed bursts produced in spheroplasts by heteroduplex $\phi_1(+)$ $\phi_0(-)$ and $\phi_6(+)$ $\phi_0(-)$ both mutations are located in gene *F* (Baas & Jansz, 1972).

ts116 is a temperature-sensitive mutant in cistron B, originally isolated and characterized in the laboratory of Dr R. L. Sinsheimer.
A distinction between ϕ_0 and *ts116* can be made by plating under the following conditions. Plates were shifted from 30 to 41°C 2·5 hr after titration. After incubation for 4 hr at 41°C, the plaques were counted. *ts116* produces tiny plaques and ϕ_0 produces large plaques.

(c) *Preparation of DNA*

Single-stranded DNA (plus-strand) of bacteriophage ϕX174 (Jansz, Van Rotterdam & Cohen, 1966) and double-stranded ϕX174 DNA (Jansz, Pouwels & Schiphorst, 1966) were prepared as previously described. The closed circular duplex (component I) of double-stranded ϕX DNA was isolated in a pure form by means of a CsCl gradient containing ethidium bromide by the procedure of Radloff, Bauer & Vinograd (1967). The closed circular duplex was converted to the nicked circular duplex (component II) by pancreatic deoxyribonuclease as previously described (Jansz, Baas, Pouwels, Van Bruggen & Oldenziel, 1968).

The minus-strand of ϕX DNA was obtained by heat denaturation for 3 min at 90°C in 0·01 M-Tris–0·001 M-EDTA (pH 8·5) of double-stranded ϕX DNA (75 μg/ml.) containing approximately equal quantities of component I and component II in the presence of the same amount of poly(U,G) (Baas & Jansz, 1971). After cooling in an ice-bath, solid CsCl was added to a density of 1·73 g/ml. The initial density was determined by refractometry. The mixture was then centrifuged in Polyallomer tubes of the 50 rotor (angle head) at 38,000 rev./min for 70 hr at 10°C in a Beckman–Spinco L2 ultracentrifuge. The solution was overlaid with liquid paraffin to prevent tube collapse. After the run, the tubes were punctured and fractions of 0·25 ml. were collected. The volume of these fractions was adjusted to 1·0 ml. with water and the absorbance at 260 nm was determined. Three components were obtained which represent double-stranded ϕX DNA component I ($\rho = 1{\cdot}706$ g/ml.), the plus-strand–poly(U,G) complex ($\rho = 1{\cdot}735$ g/ml.) and the minus-strand–poly(U,G) complex ($\rho = 1{\cdot}761$ g/ml.) (Baas & Jansz, 1971; Sheldrick & Szybalski, 1967). For further purification the fractions containing the minus-strand were pooled, and after heating for 1 hr at 65°C to anneal remaining plus-strand with minus-strand, recentrifuged to equilibrium in CsCl.

Contamination of the isolated minus-strand with plus-strand or double-stranded DNA can easily be detected by determining the u.v. survival curve, because single-stranded ϕX DNA is about 8 times more sensitive to u.v. irradiation than double-stranded ϕX DNA (Sinsheimer, Starman, Nagler & Guthrie, 1962; Jansz, Pouwels & Van Rotterdam, 1963). The u.v. survival curve of a minus-strand preparation after two successive runs in CsCl follows single-stranded u.v.-inactivation over at least three orders of magnitude and is virtually free from double-stranded DNA.

(d) *Preparation and characterization of heteroduplex replicative form*

Heteroduplex RF was prepared and characterized as previously described (Baas & Jansz, 1971). Equal amounts (5 to 10 μg/ml.) of minus-strand (after two successive runs in CsCl) obtained from wild-type (ϕ_0) RF were annealed with the same amount of plus-strand obtained from a first-step host-range mutant ϕX phage (ϕ_1) in 2 × SSC (SSC is 0·15 M-NaCl–0·015 M-sodium citrate), pH 7·0, at 65°C for 90 min. After annealing, the DNA solution was passed through a MF14 filter. This filter retains single-stranded DNA, whereas double-stranded DNA passes through (Jansz, Pouwels & Schiphorst, 1966). The resulting heteroduplex RF thus contains wild-type minus-strand and host-range-type plus-strand: $\phi_1(+)$ $\phi_0(-)$. Similarly, the heteroduplex $\phi_0(+)$ $\phi_1(-)$ and the homoduplex $\phi_0(+)$ $\phi_0(-)$ were prepared.

In control experiments the isolated minus-strand was exposed to the same annealing conditions omitting the plus-strand; less than 0·1% of the biological activity passed through the filter as compared to the above experiments.

In an analytical buoyant density CsCl gradient, homoduplex or heteroduplex RF show only one peak at the density of native double-stranded ϕX DNA ($\rho = 1{\cdot}706$ g/ml.). This indicates that these preparations contain no significant amount of single-stranded DNA ($\rho = 1{\cdot}724$ g/ml.) nor partial DNA duplex structures of intermediate density. The appearance of heteroduplex and homoduplex RF in the electron microscope, using the technique

of Kleinschmidt, is indistinguishable from that of native RF component II (Kleinschmidt, Lang, Jackerts & Zahn, 1962). The great majority of molecules are circular (length of the homoduplex $\phi_0(+)$ $\phi_0(-) = 1{\cdot}58 \pm 0{\cdot}08$ μm (28 measurements); length of the heteroduplex $\phi_1(+)$ $\phi_0(-) = 1{\cdot}55 \pm 0{\cdot}11$ μm (35 measurements)).

(e) *Analytical ultracentrifuge analyses*

Sedimentation analyses and CsCl equilibrium analyses were performed in a Spinco model E ultracentrifuge equipped with an automatic scanning device, using a double sector cell at 44·770 rev./min in a AN-D rotor. Sedimentation coefficients were determined by the moving boundary technique.

The density of the DNA was calculated using the formula of Meselson (1957). ϕX double-stranded DNA (ρ = 1·706 g/ml.) and ϕX single-stranded DNA (ρ = 1·724 g/ml.) were used as internal density markers (Pouwels, Knijnenburg, Van Rotterdam, Cohen & Jansz, 1968).

(f) *Biological activity of ϕX174 DNA*

DNA was incubated with spheroplasts of *E. coli* according to Guthrie & Sinsheimer (1960). Phage titres were determined after lysis of spheroplasts, 3 hr after the addition of DNA.

Infected spheroplasts were plated as described by Guthrie & Sinsheimer (1960).

Single-burst experiments were performed as follows: the spheroplasts were diluted in nutrient broth containing 2% bovine serum albumin 6 min after addition of DNA. From the appropriate dilution, 0·1 ml. was added to 0·5 ml. nutrient broth and incubated for 3 hr at 37°C. When DNA of a temperature-sensitive mutant of ϕX phage was used, the incubation was carried out at 30°C. After incubation, the contents of the tubes were titrated for mature ϕX phage.

(g) *Mitomycin C treatment of* E. coli *K12* hcr$^-$

Treatment of *E. coli* K12 *hcr*$^-$ with mitomycin C was carried out at 37°C in the dark for 10 min, without aeration, by adding a solution of mitomycin C (1 mg/ml.) to the culture.

After the treatment, the cells were collected by centrifugation and converted to spheroplasts.

(h) *Ultraviolet irradiation*

Ultraviolet irradiation was performed by illumination of DNA samples with a low-pressure mercury tube (Philips, 30 W, T.U.V.).

(i) *Hydroxylamine inactivation*

The incubation was carried out at 37°C, the mixture consisting of ϕX DNA in 0·1 M-sodium phosphate buffer, pH 6·0, containing 2 mM-EDTA. From a solution of 1 M-hydroxylamine, pH 6·0, hydroxylamine was added to a final concentration of 0·2 M. From time to time, samples were withdrawn and diluted 50-fold in cold 0·01 M-phosphate buffer, pH 7·0. The samples can be stored frozen for several weeks without loss of biological activity.

(j) *Materials*

CsCl *supra pur*, from Merck; ethidium bromide from J. H. Rooster (Rotterdam); several batches of poly(U,G) (ratio U:G = 1) were purchased from Miles Chemical Co. Poly (U,G) was dissolved in sterile water (1 mg/ml.) and stored at −20°C. Mitomycin C was purchased from the Sigma Chemical Co. Other materials have been described previously (Jansz, 1966; Jansz, Pouwels & Schiphorst, 1966; Jansz *et al.*, 1968).

3. Results

(a) *Biological properties of heteroduplex replicative form*

Heteroduplex RF is infectious to spheroplasts of *E. coli* K12, producing a burst of approximately equal quantities of ϕ_0 and ϕ_1 phage.

These heteroduplexes, as well as the homoduplex, are indistinguishable from native

RF, component II, in buoyant density in CsCl, in appearance in the electron microscope and in hydroxylamine sensitivity. However, the u.v. sensitivity of heteroduplex RF is intermediate between that of single-stranded and double-stranded ϕX DNA. It is unlikely that the greater u.v. sensitivity of heteroduplex RF is due to single-stranded gaps in the duplex structure, since the u.v. sensitivity of the homoduplex $\phi_0(+)\ \phi_0(-)$, which was prepared under similar conditions, equals that of native RF (Baas & Jansz, 1971).

The following experiment shows that the intermediate u.v. sensitivity is a characteristic property of heteroduplex RF and is not caused by imperfect annealing of two strands with non-matching base pairs. ϕX RF, component I, was treated with hydroxylamine. Hydroxylamine reacts with cytosine residues in DNA. The reaction product, 4-hydroxylamine-4-5-dihydroxytosine, has U-like binding properties and is considered to be responsible for replication errors in hydroxylamine mutagenesis; e.g. the conversion of a G · C base pair to an A · T base pair (Brown & Phillips, 1965). It may be expected that reaction of a cytosine residue in DNA with hydroxylamine leads to some extent to a situation in the DNA helix comparable with a heteroduplex region. The u.v. survival curves of the treated DNA samples are biphasic, indicating that the majority of the DNA molecules has intermediate u.v. sensitivity (Fig. 1).

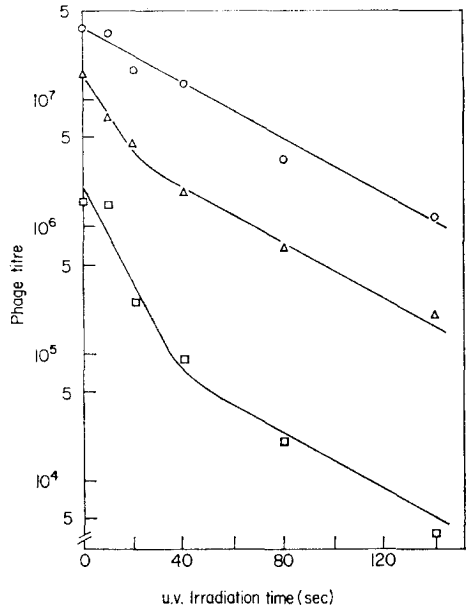

FIG. 1. Ultraviolet survival curves of native RF, component I, treated during different periods with hydroxylamine. —○—○—, Without treatment; —△—△—, after 7 hr of treatment; —□—□—, after 22 hr of treatment.

(b) *Inhibition of repair of heteroduplex replicative form in infected spheroplasts*

In a previous publication (Baas & Jansz, 1971) it was shown that in a large fraction (85%) of the infected spheroplast, heteroduplex RF is converted to homoduplex RF before replication, presumably by a mechanism comparable to that of host-cell

reactivation of u.v.-irradiated phage (Boyce & Howard-Flanders, 1964; Setlow & Carrier, 1964; Pettijohn & Hanawalt, 1964).

Doerfler & Hogness (1968) and Spatz & Trautner (1970) came to the same conclusion from similar experiments, using heteroduplex λ DNA and heteroduplex SPP1 DNA, respectively.

In our experiments a small fraction of the spheroplasts produces phage of both genotypes, suggesting that in these spheroplasts heteroduplex RF has escaped the repair process. If this interpretation is correct, the elimination of repair must increase this fraction and further analysis may provide information on the mechanism of replication of heteroduplex RF.

A number of *E. coli* strains known to be defective in repair of u.v. lesions or recombination including *E. coli* W3110 *polA1*$^-$ (De Lucia & Cairns, 1969); *E. coli* K12 *hcr*$^-$ (Harm); *E. coli* H502, *su*$^-$, *hcr*$^-$, *thy*$^-$ and Endo I$^-$ (Hofmann-Berling); *E. coli* K12 RecA13 and *E. coli* K12 RecB21 (Howard-Flanders & Theriot) and *E. coli* K12 RecC22 (Willetts & Mount) were converted to spheroplasts using the lysozyme–EDTA procedure (Guthrie & Sinsheimer, 1960) and, after infection with heteroduplex RF, the infected spheroplasts were plated on the appropriate mixed indicator. The results were essentially similar to those obtained with *E. coli* K12 or *E. coli* K58 spheroplasts. Also u.v. irradiation of *E. coli* K12 spheroplasts before infection with heteroduplex RF does not alter the above results. This suggests that mechanisms other than those involved in repair of u.v. lesions or recombination operate in the case of heteroduplex repair.

However, when *E. coli* K12 *hcr*$^-$ was treated with mitomycin C (50 μg/ml.) before the conversion to spheroplasts, quite different results were obtained (Table 1). The rationale behind this approach is that the mitomycin C lesions in host DNA compete for the enzymes that are otherwise involved in the repair of heteroduplex RF. Mitomycin C treatment of *E. coli hcr*$^-$ does not interfere with ϕX infection, although it arrests host DNA synthesis almost completely (Lindqvist & Sinsheimer, 1967). The fraction of spheroplasts producing both genotypes and the fraction of spheroplasts

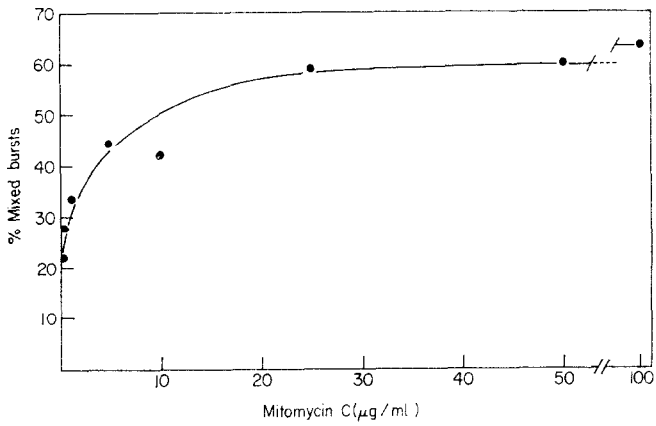

FIG. 2. The percentage of mixed bursts, produced by infection of spheroplasts of *E. coli* K12 *hcr*$^-$, treated with different amounts of mitomycin C, with $\phi_6(+)$ $\phi_0(-)$ heteroduplex RF, is plotted against the concentration of mitomycin C. The percentage of mixed bursts was determined by plating infected spheroplasts on the mixed indicator *E. coli* CO and *E. coli* CH (see Table 1).

TABLE 1

Titration of spheroplasts infected with heteroduplex replicative form

Heteroduplex	Spheroplasts of *E. coli*	Number of plaques scored	Plaques of plus-strand type	Plaques of minus-strand type	Mixed plaques
$\phi_1(+)\ \phi_0(-)$	K12 *hcr*$^+$	70	33 (48%)	28 (40%)	9 (12%)
	K12 *hcr*$^-$	209	30 (14%)	125 (60%)	54 (26%)
	Treated with 50 μg mitomycin C/ml.				
$\phi_0(+)\ \phi_1(-)$	K12 *hcr*$^+$	425	321 (75%)	86 (20%)	18 (5%)
	K12 *hcr*$^-$	305	94 (31%)	141 (46%)	70 (23%)
	Treated with 50 μg mitomycin C/ml.				
$\phi_6(+)\ \phi_0(-)$	K12 *hcr*$^+$	135	66 (49%)	38 (27%)	31 (24%)
	K12 *hcr*$^-$	132	19 (14%)	38 (29%)	75 (57%)
	Treated with 50 μg mitomycin C/ml.				

ϕ_0 is wild-type ϕX174. ϕ_1 and ϕ_6 are different host-range mutants of ϕX174 in cistron F. Spheroplasts infected with heteroduplex RF were plated on a mixed indicator (*E. coli* CO and *E. coli* C1 for $\phi_1(+)\ \phi_0(-)$ and $\phi_0(+)\ \phi_1(-)$ heteroduplex RF and *E. coli* C0 and *E. coli* CH for $\phi_6(+)\ \phi_0(-)$ heteroduplex RF). In this way a distinction can be made between single and mixed bursts. A burst of only wild-type phage yields a turbid plaque. A burst of only host-range phage yields a clear plaque. A mixed burst yields a turbid plaque with a clear centre.

producing only phage of the minus-strand genotype increased, whereas the fraction producing phage of the plus-strand genotype decreased. The relation between the increase of the fraction of spheroplasts, producing both genotypes and the concentration of mitomycin C is shown in Figure 2. There is a threefold increase of the fraction of spheroplasts producing both genotypes, and the effect of mitomycin C levels off at a concentration of 25 μg/ml.

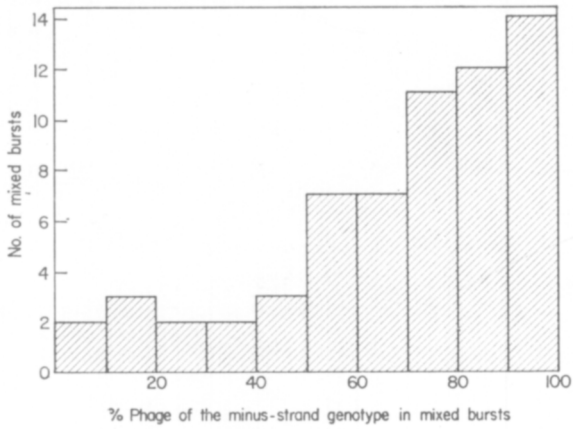

FIG. 3. Distribution of phage of the minus-strand genotype in mixed bursts. 6 min after addition of $\phi_6(+)$ $\phi_0(-)$ heteroduplex RF, the *E. coli* K12 *hcr*$^-$ spheroplasts, treated with 50 μg mitomycin C/ml., were diluted to 0·25 infective centre/test tube and incubated for 3 hr at 37°C. After incubation; the tubes were titrated for mature ϕX phage. 63 mixed bursts were analysed. The number of bursts with the percentage of phage of the minus-strand genotype, within the limits indicated, is plotted against the percentage of the minus-strand genotype.

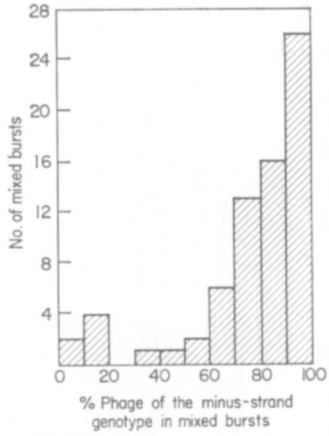

FIG. 4. Distribution of phage of the minus-strand genotype in mixed bursts. 6 min after addition of *ts116*(+) $\phi_0(-)$ heteroduplex RF, the *E. coli* K58 spheroplasts were diluted to less than 0·5 infective centre/test tube and incubated for 3 hr at 30°C. After incubation, the tubes were titrated for mature ϕX phage. 71 mixed bursts were analysed. The number of bursts with the percentage of phage of the minus-strand genotype, within the limits indicated, is plotted against the percentage of the minus-strand genotype.

(c) *Analysis of the phage produced in mixed bursts*

The increase in the percentage of spheroplasts producing both genotypes, caused by mitomycin C treatment, allowed us to analyse the proportion of phage of the minus-strand genotype to that of the plus-strand genotype occurring in mixed bursts. In a single-burst experiment, it was shown that in mixed bursts the majority of the phage has the genotype of the minus-strand (Fig. 3).

As shown in the accompanying paper (Baas & Jansz, 1972) the size of the fraction of infected spheroplasts which produce both genotypes depends on the cistron in which the heteroduplex region is located. This allows an analysis of this fraction in the absence of mitomycin C. A single-burst analysis of *ts116*(+) $\phi_0(-)$ heteroduplex RF (*ts116* is located in cistron B) again indicates that in mixed bursts (32%) the majority of the phage has the genotype of the minus-strand (Fig. 4). It is concluded that in spheroplasts, where replication of heteroduplex RF takes place, the minus-strand is the major information precursor for progeny phage.

4. Discussion

The DNA replication of the bacteriophage ϕX174 occurs in two phases: a period of synthesis of double-stranded circular DNA, followed by a period of single-strand circular DNA synthesis. In the second phase of DNA replication the first phase is shut off. Experiments of Gilbert & Dressler (1968), Dressler & Wolfson (1970), Knippers, Razin, Davis & Sinsheimer (1969) and Knippers, Whalley & Sinsheimer (1969) have shown that in both phases the DNA is replicated according to the rolling circle model. In the phase of single-stranded DNA synthesis, the minus-strand remains circular and the plus-strand is elongated in the $5' \rightarrow 3'$ direction. Full ϕX genomes are peeled off, circularized and packaged into coat protein. However, the results of the experiments of Knippers, Whalley & Sinsheimer (1969) concerning the mechanism of the DNA replication of the double-stranded ϕX DNA are in contradiction with the results of Dressler & Wolfson (1970). Knippers, Whalley & Sinsheimer (1969) propose a rolling circle in which the plus-strand remains circular and the minus-strand is elongated. Dressler & Wolfson (1970) propose a rolling circle in which the minus-strand remains circular and the plus-strand is elongated. As a consequence, the first model predicts that the parental plus-strand is the master template in ϕX RF replication and in the second model the parental minus-strand fulfils this function.

To obtain more information on the mechanism of replication of ϕX RF we have prepared heteroduplex RF in which the plus- and minus-strand each contain a different genetic marker. Spheroplasts of the appropriate host were infected with this heteroduplex RF and the induced progeny phage was analysed. Assuming that most, if not all, of the progeny RF molecules, synthesized in the first phase of ϕX DNA replication, participate equally in the synthesis of single-stranded phage DNA, an analysis of the proportion of the genotypes of the phage produced in single bursts reflects the proportion in the genotypes of the RF pool. This analysis might indicate whether the plus- or the minus-strand is the master template during RF replication.

However, the finding that the majority (85%) of the spheroplasts infected with heteroduplex RF $\phi_0(+)$ $\phi_1(-)$ produces either phage of the plus- or phage of the minus-strand genotype is hard to reconcile with any plausible replication mechanism. Also the fact that *em25*(+) $\phi_1(-)$ heteroduplex RF produces a high percentage of ϕ_0 phage strongly argues for a repair process of heteroduplex RF to homoduplex RF before replication (Baas & Jansz, 1971).

The higher u.v. sensitivity of heteroduplex RF, as compared to homoduplex RF prepared under similar conditions, can be the result of the repair process. The u.v. sensitivity is the only difference in physical and biological properties that has been found between heteroduplex and homoduplex RF. In contrast to homoduplex RF, the u.v. sensitivity of heteroduplex RF is more like single-stranded than double-stranded ϕX DNA. This suggests that repair of a heteroduplex region involves the degradation of a large single-strand segment. Excision of a large and specific segment of one strand in u.v.-irradiated heteroduplex RF, results in a DNA duplex with a single-stranded region. In this single-stranded region u.v. lesions are lethal, since they cannot be repaired by excision and repair synthesis. As a consequence, the sensitivity of heteroduplex RF to u.v. irradiation is increased as compared to native RF or homoduplex RF.

The fact that a fraction (10 to 15%) of the spheroplasts produces plus-strand as well as minus-strand type phage, suggests that heteroduplex RF in these spheroplasts has escaped the repair process. This fraction is not caused by the infection of a spheroplast by more than one heteroduplex RF molecule because the concentration of DNA used in the experiments was low. Also a further dilution of the DNA to the limit of detection does not alter the results. In a control experiment, it was shown that to produce mixedly infected spheroplasts with a mixture of RF ϕ_0 and RF ϕ_1 requires a 100-fold higher DNA concentration than that used in the present experiments. Also the fact that the fraction of mixed bursts is dependent on the cistron where the heteroduplex region is located is a strong argument for the validity of the present conclusions.

Following our initial objective, we have looked for conditions in which the repair process can be eliminated. We succeeded in this purpose by pretreatment of spheroplasts of *E. coli* K12 *hcr*$^-$ with mitomycin C. The result of the mitomycin C treatment is an increase of spheroplasts, producing phage of both genotypes and an increase of spheroplasts, producing phage of minus-strand genotype. In single-burst experiments, it was shown that the majority of the phage in mixed bursts has the genotype of the minus-strand. Similar results were obtained in the absence of mitomycin C using *ts116* (+) ϕ_0(−) heteroduplex RF with a heteroduplex region in cistron B.

These experiments clearly show that, under conditions where replication of heteroduplex RF can take place, the minus-strand of heteroduplex RF is the major information precursor for the DNA of progeny phage. Also these results offer an interpretation of similar experiments performed by Merriam, Dumas & Sinsheimer (1971) and Merriam Funk & Sinsheimer (1971). This suggests an asymmetric mechanism of RF replication, in which the minus-strand is used several times as a master template in the formation of progeny RF molecules, as predicted by the rolling circle model of Dressler & Wolfson (1970). We interpret our results as follows: after infection of a spheroplast with heteroduplex RF, there is a competition between repair and replication of heteroduplex RF. Further evidence for a competition between repair and replication of heteroduplex RF is given in the accompanying paper (Baas & Jansz, 1972). Most of the heteroduplexes are converted to homoduplexes before replication can start. The result of the repair process is a spheroplast, yielding either phage of the plus- or phage of the minus-strand genotype. If heteroduplex RF escapes the repair process, the first cycle of semiconservative DNA replication produces two homoduplexes, one of the minus-strand type and one of the plus-strand type. The homoduplex of the minus-strand type governs most of the further RF replication, producing

a pool of progeny RF molecules from which progeny phage DNA is derived.

To account for the results of Knippers & Sinsheimer (1968), it is proposed that the homoduplex of the plus-strand type also replicates but with low probability, explaining the minor fraction of plus-strand type phage in spheroplasts, which produce both types of phages. However, the major function of this RF molecule might be to provide for transcription and regulation during phage development, which requires that the integrity of this molecule is preserved (Denhardt & Sinsheimer, 1965).

An alternative explanation of these results is that RF does not replicate in spheroplasts and that the progeny phage DNA is all derived from the infecting (parental) RF. In this case progeny phage of the minus-strand genotype would be expected as the major product of single bursts. However, this possibility seems highly unlikely, if not excluded, for the following reasons.

(1) The burst size of mixed bursts is comparable to that of ϕX-infected cells. In the single-burst experiments described a mean burst size of 74 was found for ϕ_6 (+) ϕ_0 (−) heteroduplex RF and a mean burst size of 117 for *ts116* (+) ϕ_0 (−) heteroduplex RF.

(2) ϕX DNA of mutants in cistron A, the product of which is necessary for RF replication, produces no progeny phage in spheroplasts under restrictive conditions, indicating that RF replication is obligatory for phage production.

(3) If there is no RF replication in spheroplasts, spheroplasts infected with heteroduplex RF will yield progeny phage all but one of which are of the minus-strand genotype. However, the great majority of mixed bursts, even in the region where phage of the minus-strand genotype contributes 90% of the total burst, contains more than one phage of the plus-strand genotype. Also a limited RF replication producing one or two progeny RF with the plus-strand as the master template does not agree with the present results. In this case a symmetrical distribution of progeny phage in mixed bursts would be expected.

This work was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

The excellent technical assistance of Miss J. Woutersen and Mrs R. Kousbroek is gratefully acknowledged.

REFERENCES

Baas, P. D. & Jansz, H. S. (1971). *Proc. Roy. Neth. Acad. Sci.* C,74, **2**, 191.
Baas, P. D. & Jansz, H. S. (1972). *J. Mol. Biol.* **63**, 567.
Boyce, R. P. & Howard-Flanders, P. (1964). *Proc. Nat. Acad. Sci., Wash.* **51**, 293.
Brown, D. M. & Phillips, J. H. (1965). *J. Mol. Biol.* **11**, 663.
De Lucia, P. & Cairns, J. (1969). *Nature*, **224**, 1164.
Denhardt, D. T. & Sinsheimer, R. L. (1965). *J. Mol. Biol.* **12**, 663.
Doerfler, W. & Hogness, D. S. (1968). *J. Mol. Biol.* **33**, 661.
Dressler, D. & Wolfson, J. (1970). *Proc. Nat. Acad. Sci., Wash.* **67**, 456.
Gilbert, W. & Dressler, D. (1968). *Cold Spr. Harb. Symp. Quant. Biol.* **33**, 473.
Guthrie, G. D. & Sinsheimer, R. L. (1960). *J. Mol. Biol.* **2**, 297.
Jansz, H. S., Baas, P. D., Pouwels, P. H., Van Bruggen, E. F. J. & Oldenziel, H. (1968). *J. Mol. Biol.* **32**, 159.
Jansz, H. S., Pouwels, P. H. & Schiphorst, J. (1966). *Biochim. biophys. Acta*, **123**, 626.
Jansz, H. S., Pouwels, P. H. & Van Rotterdam, C. (1963). *Biochim. biophys. Acta*, **76**, 655.
Jansz, H. S., Van Rotterdam, C. & Cohen, J. A. (1966). *Biochim. biophys. Acta*, **119**, 276.
Kleinschmidt, A. K., Lang, D., Jackerts, D. & Zahn, R. K. (1962). *Biochim. biophys. Acta*, **61**, 857.

Knippers, R., Komano, T. & Sinsheimer, R. L. (1968). *Proc. Nat. Acad. Sci., Wash.* **59**, 577.

Knippers, R., Razin, A., Davis, R. & Sinsheimer, R. L. (1969). *J. Mol. Biol.* **45**, 239.

Knippers, R. & Sinsheimer, R. L. (1968). *J. Mol. Biol.* **34**, 17.

Knippers, R., Whalley, J. M. & Sinsheimer, R. L. (1969). *Proc. Nat. Acad. Sci., Wash.* **64**, 275.

Komano, T., Knippers, R. & Sinsheimer, R. L. (1968). *Proc. Nat. Acad. Sci., Wash.* **59**, 911.

Lindqvist, B. H. & Sinsheimer, R. L. (1967). *J. Mol. Biol.* **30**, 69.

Lindqvist, B. H. & Sinsheimer, R. L. (1968). *J. Mol. Biol.* **32**, 285.

Merriam, V., Dumas, L. B. & Sinsheimer, R. L. (1971). *J. Virol.* **7**, 603.

Merriam, V., Funk, F. & Sinsheimer, R. L. (1971). *Mutation Res.* **12**, 206.

Meselson, M. (1957). Thesis, California Institute of Technology.

Pettijohn, D. & Hanawalt, P. (1964). *J. Mol. Biol.* **9**, 395.

Pouwels, P. H., Knijnenburg, C. M., Van Rotterdam, J., Cohen, J. A. & Jansz, H. S. (1968). *J. Mol. Biol.* **32**, 169.

Radloff, R., Bauer, W. & Vinograd, J. (1967). *Proc. Nat. Acad. Sci., Wash.* **57**, 1514.

Setlow, R. B. & Carrier, W. L. (1964). *Proc. Nat. Acad. Sci., Wash.* **51**, 226.

Sheldrick, P. & Szybalski, W. (1967). *J. Mol. Biol.* **29**, 217.

Sinsheimer, R. L. (1968). *Progress in Nucleic Acid Research and Molecular Biology*, vol. 8, p. 115, New York: Academic Press.

Sinsheimer, R. L., Starman, B., Nagler, C. & Guthrie, S. (1962). *J. Mol. Biol.* **4**, 142.

Spatz, H. Ch. & Trautner, T. A. (1970). *Molec. Gen. Genetics,* **109**, 84.