Eur. J. Biochem. *119*, 663–668 (1981)

Bacteriophage fd Gene-2 Protein

Processing of Phage fd Viral Strands Replicated by Phage T7 Enzymes

Günter HARTH, Irmtraud BÄUMEL, Thomas F. MEYER, and Klaus GEIDER

Max-Planck-Institute for Medical Research, Department of Molecular Biology, Heidelberg

(Received June 9, 1981)

Bacteriophage T7 gene 4 protein and DNA polymerase of the phage were used to study the viral strand synthesis of bacteriophage fd *in vitro*. Cleavage of supercoiled phage fd replicative form (RF) by fd gene 2 protein produced a nick at a specific site in the viral strand. The cleaved double-stranded DNA was unwound by T7 gene 4 protein and T7 DNA polymerase and the 3′ end of the nicked strand simultaneously extended according to the rolling circle mechanism. After a complete round of DNA synthesis fd gene 2 protein cleaved the viral strand presumably at the same site, where the endonuclease cuts fd RF I, and subsequently sealed the single-stranded linear DNA into a circle. The reaction products were analyzed by velocity sedimentation, gel electrophoresis and electron microscopy. Most of the single-stranded DNA synthesized was circular. No host proteins were required for the formation of the single-stranded circles. Strand switching of the T7 DNA polymerase indicated by double-stranded tails of the rolling circle structures reduced the yield of viral single-stranded circles in this enzyme system.

Phage fd gene 2 protein is essential for viral strand synthesis of phage fd [1,2]. The protein has been purified [3] and shown to cleave supercoiled fd RF in the viral strand [4] between nucleotides 5781 and 5782 [5]. Gene 2 protein does not only cleave fd RF I, but it is also able to seal the nicked strand after relaxation of the RF [4]. This joining activity is assumed to be involved in circularization of the viral strands after cleavage of the displaced single strands following a round of rolling circle replication.

Phage fd RF can be replicated *in vitro* using the purified *Escherichia coli* proteins DNA polymerase III holoenzyme, *rep* protein and DNA binding protein I in the presence of phage fd gene 2 protein [6]. T7 DNA polymerase and T7 gene 4 protein are another powerful system for strand unwinding and for replication of double-stranded DNA *in vitro* according to the rolling circle mechanism, as was shown for nicked PM2 DNA [7]. Another function of T7 gene 4 protein is the synthesis of priming oligonucleotides, thus allowing, in the presence of T7 DNA polymerase, replication on single-stranded DNA. Phage ϕX174 circular single strands as template were replicated by the T7 enzymes for several rounds [8]. We could show that phage fd RF provides a substrate for replication with these enzymes after cleavage by fd gene 2 protein. In contrast to ϕX174 gene A protein, it was found that gene 2 protein does not bind tightly to the cleaved DNA [4]. After one round of DNA replication the replicated viral strands were found to be cleaved by gene 2 protein in linear molecules of unit length, which were subsequently circularized by the enzyme.

T7 DNA polymerase has a high tendency to switch from the leading strand to the lagging strand [7,9]. This event interferes with the viral strand synthesis of the rolling circles.

Abbreviations. RF, circular double-stranded DNA of replicative form; RF I, covalently closed, supercoiled RF; RF II, RF with a discontinuity is the viral strand; RF IV, covalently closed, relaxed RF; dBTP, 5-bromo-2′-deoxyuridine 5′-triphosphate.

MATERIALS AND METHODS

Materials

Escherichia coli strains: B4 is *endA*100, *str*; BBw/1 is *supE* (from Dr R. Hausmann); H570 *ton* 4 is *polA1*, *endA2*, *rnsA100*, *tsx*, *str*, *ton*.

Phages: T7 am3 am6 (from Dr R. Knippers) has amber mutations in gene 3 and in gene 6. The double mutant was grown in BBw/1 and tested for reversions in the mutated genes by complementation with appropriate T7 mutants.

The sources of DNA and nucleotides have been described previously [3].

Enzymes

Phage fd gene 2 protein was prepared as described [3]. T7 DNA polymerase and T7 gene 4 protein were isolated from H570 *ton* 4 cells infected with phage T7 am3 am6 by modifying published procedures [8,10]. The polymerase was about 80% pure as judged by polyacrylamide gel electrophoresis and had a specific activity of 10000 units/mg protein. One unit is the amount catalyzing the incorporation of 1 nmol deoxynucleotides in 10 min at 37 °C into denatured calf thymus DNA. Gene 4 protein was about 60% pure and had a specific activity of 2000 units/mg protein. One unit is defined as the amount stimulating T7 DNA polymerase to incorporate 1 nmol deoxynucleotides in 30 min at 30 °C using phage fd single-stranded circles as template. The absence of contaminating DNase and DNA ligase activities was demonstrated by incubating fd RF I (200 ng) and nicked fd RF (150 ng), respectively, with the polymerase or the gene 4 protein preparations in the presence of Mg^{2+}. For the ligase assays ATP was also added. The DNA was separated by gel electrophoresis in 0.7% agarose. To separate RF IV and RF II, ethidium bromide was added to 1 μg/ml. No cleavage of RF I or sealing of RF II was observed, when preparations of T7 DNA

polymerase (6 units) or T7 gene 4 protein (0.5 unit) were present.

Replication Assay

Phage fd RFI (100 ng) was incubated at 30 °C with 0.16 unit of fd gene 2 protein, 0.2 unit of T7 gene 4 protein, and 6 units of T7 DNA polymerase in 20 µl of 20% sorbit/20 mM Tris/Cl, pH 7.5, 5 mM $MgCl_2$, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, dATP, dCTP, dGTP, dTTP (100 µM each, label as [^{3}H]dTTP; 700 counts min^{-1} $pmol^{-1}$). The incubation time of samples for sedimentation and electron microscopic analysis was 20 min.

Physical Methods

Sucrose velocity sedimentation and isopycnic centrifugation in alkaline Cs_2SO_4 were carried out as described [11, 12]. Spreading of the DNA for electron micrograph pictures was done according to an established procedure [13]. The grids were investigated in a Phillips EM 400 electron microscope at magnifications between 8350 and 14000 at 40 kV.

RESULTS

Replication of fd RF with fd Gene 2 Protein and T7 Enzymes

Phage T7 gene 4 protein umwinds the strands in conjunction with T7 DNA polymerase using the dephosphorylation of dATP or ATP as an energy source [14]. These two enzymes were used to replicate phage fd RF nicked by fd gene 2 protein. As listed in Table 1, fd gene 2 protein, T7 DNA polymerase and T7 gene 4 protein were required for a maximal level of DNA synthesis. The incorporation of deoxynucleoside triphosphates was not affected by rifampicin, an inhibitor of bacterial RNA polymerase, which initiates ssDNA to dsDNA conversion of filamentous phages [11, 15], nor by addition of ribonucleoside triphosphates which are used for primer formation by T7 gene 4 protein. The rate of DNA synthesis is high within the first 20 min of incubation, and levels off around 30 min (Fig. 1). Investigation of the replication products (see below) showed a maximum of single-stranded DNA at 20 min of incubation. Later most of the replicated DNA was found to be partially double-stranded. At that stage the DNA was therefore not suitable for analysis of the circularization reaction.

Sedimentation Profiles of the Replicated DNA

After 20 min of replication the products were analyzed by velocity sedimentation in neutral sucrose and 1 M salt. Single-stranded linear and circular fd DNA sediment ahead of the double-stranded forms. Not only parental ^{3}H label, but also newly synthesized ^{32}P label were found in the position of single-stranded DNA (Fig. 2). This demonstrates that the T7 enzymes synthesized at least two rounds of fd viral strands, and gene 2 protein cleaved the DNA after replication of one genome length. At 20 min of incubation about 16% of the template were replicated and about 8% of the total DNA were found in the position of single-stranded material. Comparison of parental ^{3}H label and new ^{32}P label banding as single-stranded DNA species shows that the first round of replication is about four times more efficient than the second round.

Table 1. *Requirements for replication of fd RF with T7 enzymes and fd gene 2 protein*
Incubation was for 20 min as described under Materials and Methods (complete sytem)

System	DNS synthesis (nucleotides incorporated)
	pmol
Complete system	38
– fd RF	1
– T7 gene 4 protein	8
– T7 DNA polymerase	1
plus rNTPs (250 µM each)	41
plus rifampicin (20 µg/ml)	40

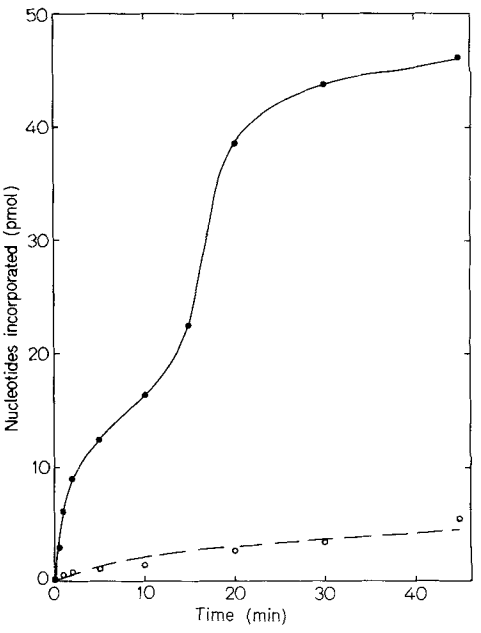

Fig. 1. *Kinetics of DNA synthesis.* The system was the complete system of Materials and Methods. Of a 200-µl assay 20-µl aliquots were acid-precipitated at the times indicated. (●——●) Complete system; (○——○) complete system without gene 2 protein

To estimate the percentage of circles and linear DNA among the single-stranded molecules, the fractions with single-stranded DNA were sedimented in alkaline sucrose and were also examined by electron microscopy. In alkali at least two-thirds of the labeled DNA sedimented at the position of circles and not more than one-third as a slower tail in the position of unit-length linear DNA (Fig. 3A). Both DNA species differ in sedimentation by 2 S (Fig. 3B). Analysis in the electron microscope confirmed that most of the DNA in the peak of Fig. 3A was of single-stranded circular structure (data not shown). These results agree with the analysis of the replication products on agarose gels.

Density Banding of Single-Stranded Material in Alkaline Cs_2SO_4

As shown in Fig. 1, gene 2 protein is responsible for the onset of DNA replication on fd RF I with T7 gene 4 protein and T7 DNA polymerase. It was demonstrated elsewhere

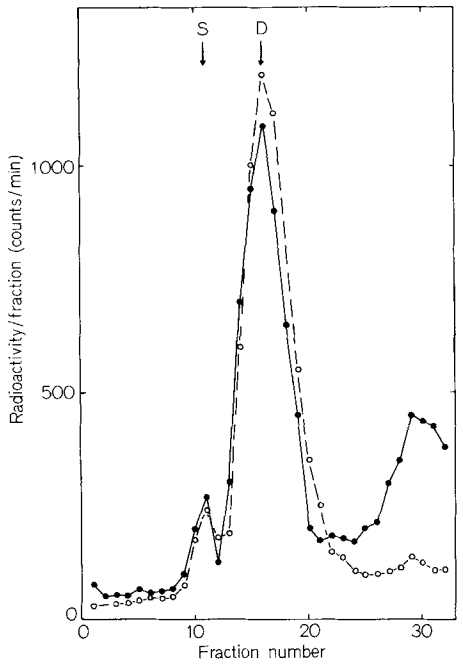

Fig. 2. *Velocity sedimentation of replication products in neutral sucrose.* ^{3}H-labeled fd RF I (O – – O; 50 ng, 10000 counts/min) replicated in the complete system (vol. 50 µl). Label was introduced from [α-^{32}P]dCTP (●——●; 1220 counts min^{-1} pmol^{-1}). After 20 min at 30 °C, NaCl and EDTA were added to 1 M and 50 mM, respectively, and the incubation mixture diluted in 100 µl 1 M NaCl, 20 mM Tris/Cl, pH 7.5, 20 mM EDTA. This mixture was layered on top of a neutral sucrose gradient (four layers with 5%, 10%, 15% and 20% sucrose, respectively, containing 1 M NaCl, 20 mM Tris/Cl, pH 7.5, 20 mM EDTA) and sedimented in a Beckman SW60 Ti rotor for 90 min ar 60000 rev./min at 20 °C. The direction of sedimentation is from right to left. Aliquots of 10 µl from each fraction were acid-precipitated and counted. Recovery of label was about 90%. The markers were circular single-stranded ^{14}C-labeled fd DNA (2000 counts/min; arrow S) and ^{3}H-labeled fd RF II (3000 counts/min; arrow D), which were run in separate tubes

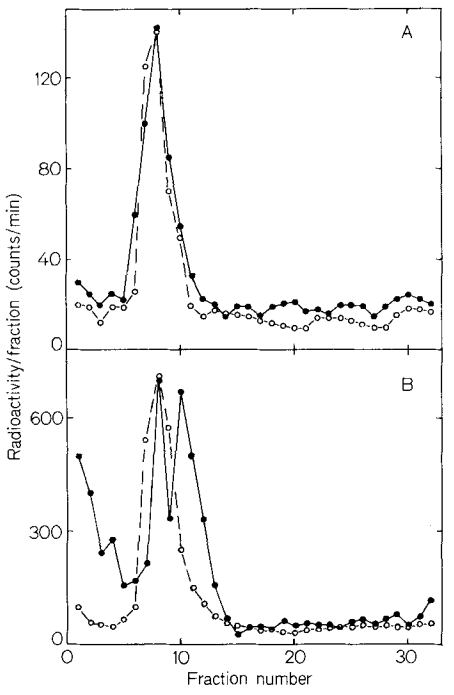

Fig. 3. *Separation of circles and linears by velocity sedimentation in alkali.* (A) Fractions 10 and 11 of Fig. 2 were dialysed against 10 mM Tris/Cl, pH 7.5, 1 mM EDTA. Before centrifugation the sample was brought to 0.3 M NaOH and 0.8 M NaCl. The solution (about 200 µl) was layered on top of an alkaline sucrose gradient (layers with 5%, 10%, 15%, 20% sucrose in 0.3 M NaOH, 0.8 M NaCl, 2 mM EDTA). Sedimentation was performed in a Beckman SW60 Ti rotor for 180 min at 60000 rev./min and 20 °C. (B) The external markers were circular single-stranded ^{14}C-labeled fd DNA (2000 counts/min; O----O) and ^{3}H-labeled fd RF II made by cleavage of fd ^{3}H-labeled RF I (4000 counts/min; ●— —●) with fd gene 2 protein. In alkali, the complementary strand sediments as single-stranded circle, the viral strand as single-stranded linear DNA

[4,5] that gene 2 protein exclusively cleaves fd RF I in the viral strand. To confirm these results for DNA replication by gene 2 protein and the T7 enzymes, DNA replication was allowed in the presence of 5-bromo-2′-deoxyuridine 5′-triphosphate (dBTP) instead of dTTP. The single-stranded material of a neutral sucrose gradient was made alkaline and banded to equilibrium in an alkaline Cs_2SO_4 gradient (Fig. 4). The incorporated radioactive label mainly banded in the position of viral strands with heavy density. Because the viral strand contains more thymidine than the complementary strand both strands can be well separated in a Cs/salt density gradient [12]. Only minute amounts of label banded in the position of fd complementary strands with heavy density. This shows that DNA replication predominantly occurs as viral strand DNA synthesis.

Agarose Gel Electrophoresis of the Replication Products

Another method to separate single-stranded circular from linear DNA is electrophoresis of the replication products on agarose gels. The double-stranded material migrated slowly on those gels (Fig. 5). On the autoradiograph a strong band in the position of single-stranded circles and a weak band in the position of unit length single-stranded linear DNA revealed that circles were generated approximately in

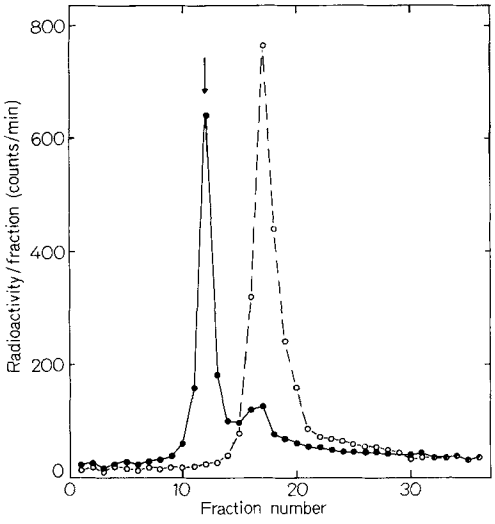

Fig. 4. *Density banding in alkaline Cs_2SO_4.* In a standard incubation assay 100 ng fd RF was replicated for 20 min in the presence of dBTP instead of dTTP. The label incorporated was [α-^{32}P]dCTP (●——●; 2400 counts min^{-1} pmol^{-1}). The reaction products were sedimented in neutral sucrose (see Fig. 2) and the fractions containing the single-stranded DNA were pooled, dialyzed, and brought to 0.1 M NaOH. Banding in alkaline Cs_2SO_4 [12] was done for 66 h at 40000 rev./min and 20 °C in a Beckman 50 Ti rotor. Internal reference was fd complementary strand (O – – O; 250 counts/min), which was synthesized *in vitro* in the presence of dBTP and [^{3}H]dATP [11]. External marker (arrow) was fd viral strand, synthesized in the presence of dBTP and [α-^{32}P]dCTP by fd RF replication with gene 2 protein and *E. coli* enzymes [6]

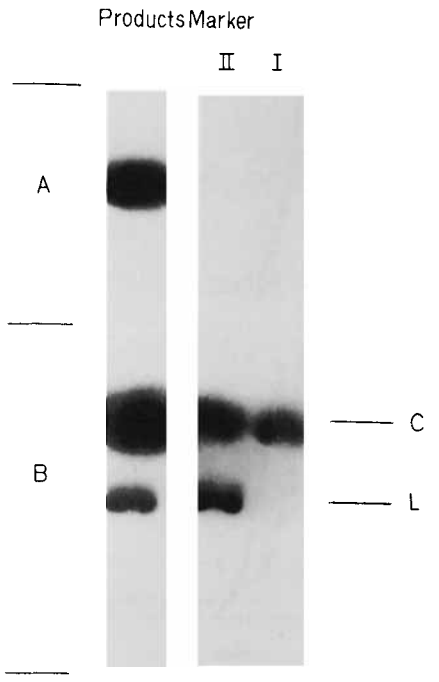

Fig. 5. *Agarose gels of the replication products.* Standard incubation with [α-^{32}P]dCTP (6600 counts min^{-1} pmol^{-1}) as labeled nucleotide was done for 20 min in a volume of 20 µl. The reaction was stopped by adding 15 µl of 60% glycerol, 50 mM EDTA, 0.2% sodium dodecyl sarcosinate, 0.1% bromophenol blue. The sample was applied to a 1.2% agarose gel (18 × 18 × 0.3 cm) and run for 22 h at 40 V. After drying the gel was exposed on a Kodak X-Omat XR-5 film. The upper part of the gel with fd RF was exposed for 3 h (A), the lower part of the gel with the single-stranded DNA for 3 days (B). The markers were single-stranded circles (I) and a mixture (II) of circular and linear ^{32}P-labeled fd DNA (5000 counts/min). The latter species was obtained by cleavage of ^{32}P-labeled phage fd single strands with pancreatic DNase. The enzyme was inactivated by brief boiling. The markers were added to the standard incubation mixture and immediately applied to the gel. The position of circular (C) and linear (L) single strands is indicated

a 3:1 ratio fitting well to the data obtained by sedimentation analysis.

Product Analysis in the Electron Microscope

The products of a standard reaction were investigated in the electron microscope. Typical structures are shown in Fig. 6. A quantitative evaluation of 371 molecules is given in Table 2,A. Besides RF as main class of molecules, 6% of the investigated molecules were replication intermediates of a rolling circle structure, whereas 10% were found as single-stranded or partially single-stranded circles or as linear DNA of unit length. Rolling circles consist of RF with a single-stranded tail (Fig. 6a). Single-stranded linear DNA of unit length and circular DNA molecules are shown in Fig. 6b and c, respectively. They were the major DNA species seen in the electron microscope when the replication products were sedimented in neutral sucrose and the fractions with the single-stranded DNA were investigated (Table 2, column B). Single-stranded fd DNA is considered as an end product of replication. The rolling circle structures were cleaved by the action of gene 2 protein, and the linear DNA was in part subsequently circularized. A few single-stranded molecules were of double or multiple genome length (Fig. 6e). Apparently they were not cleaved by gene 2 protein.

Table 2. *Electron microscope analysis of replication products*
Column A represents molecules form a standard incubation after 20 min of replication (Materials and Methods). The percentage of total number evaluated (371) is given. Column B is material from fractions 10 and 11 of Fig. 2. The percentage of total number evaluated (253) is given. No single-stranded DNA was observed in the preparation of fd RF I used as template (more than 100 molecules investigated)

Product	Amount in	
	A	B
	%	
Single-stranded DNA		
Single-stranded circles	1	39
Partially double-stranded circles	4	–
Single-stranded linear DNA of unit length	1	27
Partially double-stranded linear DNA of unit length	3	–
Single-stranded linear DNA of multiple length	1	8
Replication intermediates		
Rolling circles with single-stranded tails	1	2
Rolling circles with double-stranded tails	5	8
Double-stranded circles (RF)	84	14

As listed in Table 2 many of the DNA molecules were partially double-stranded (Fig. 6f). They might be derived from originally single-stranded DNA which was then primed by T7 gene 4 protein or by contaminating oligonucleotides. An unknown number of molecules might have been completely converted into double-stranded DNA, which cannot be distinguished in the electron microscope from the template RF II at the start or at the end of replication.

Most of the rolling circles with double-stranded tails (Fig. 6d) are probably produced by template switching of the T7 DNA polymerase [7]. Recently it was shown that fd gene 2 protein causes a delay of replication before cleavage of the displaced single-stranded tail [18]. This lag period might enhance the switch of T7 DNA polymerase from the leading strand to the displaced viral strand, thus interfering with the cleavage and circularization reaction of gene 2 protein.

Processing of Replicating Intermediates by Gene 2 Protein

Phage fd RF I was cleaved with fd gene 2 protein, and the protein was heat-inactivated or removed by phenol extraction in other experiments. Addition of T7 gene 4 protein and T7 DNA polymerase results in DNA synthesis. The products sedimented near the position of relaxed double strands (Fig. 7A). When fd gene 2 protein was also added, free single strands were observed (Fig. 7B). They sedimented in the position of circles under alkaline conditions (data not shown). This shows that the molecule of gene 2 protein which cleaves RF I can be substituted by another molecule for processing of the single-stranded tail. The energy of the latter cleavage reaction is thought to be transferred to the circularization step by the same molecule of gene 2 protein [16].

As fd gene 2 protein, T7 gene 4 protein and T7 DNA polymerase are sufficient for circle formation, it can be concluded that proteins from *Escherichia coli* do not directly participate in cleavage and circularization of the viral strands in phage fd replication.

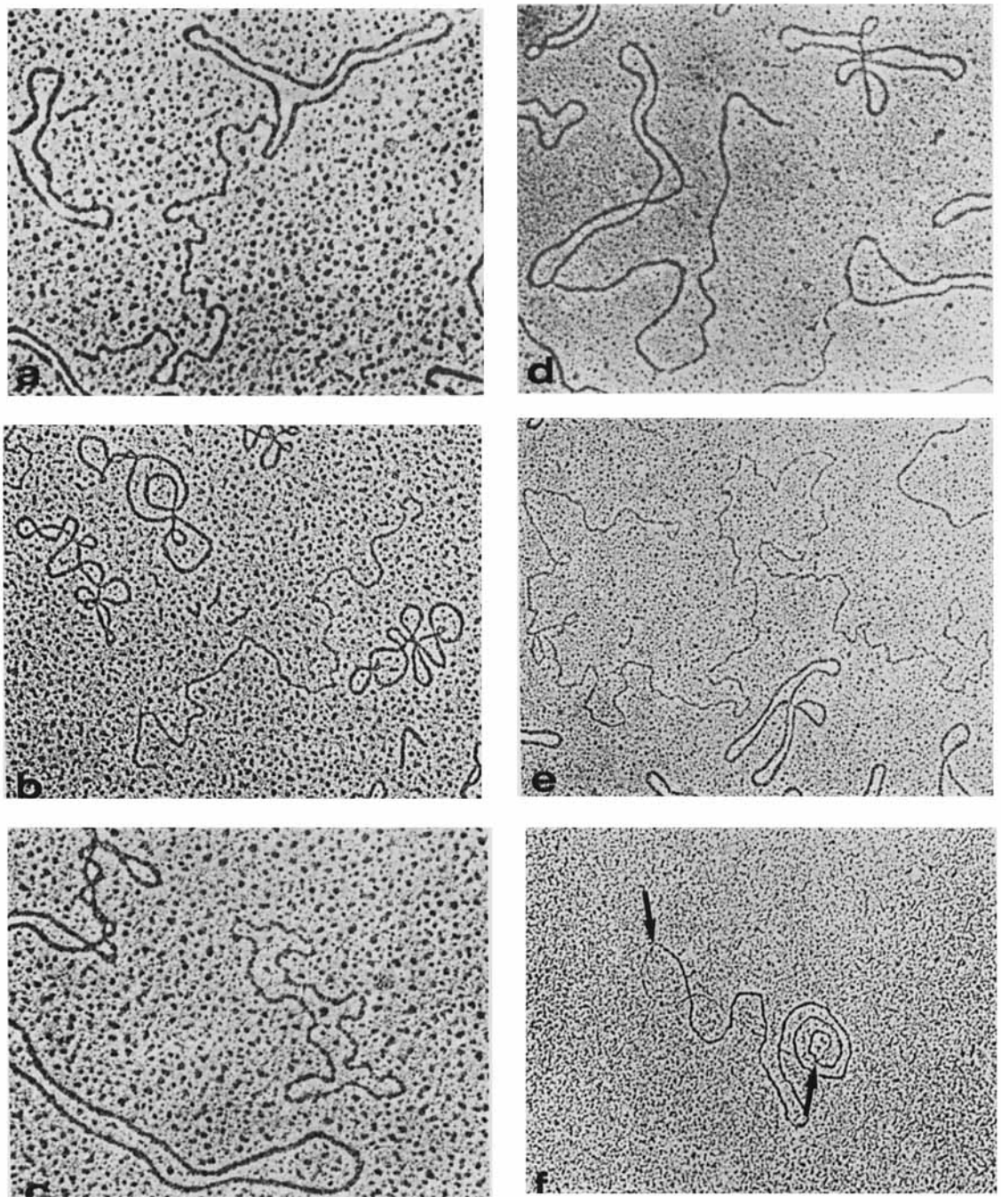

Fig. 6. *Electron micrographs of fd RF DNA replicated with phage T7 enzymes after cleavage with phage fd gene 2 protein.* DNA was incubated for 20 min and spread as described under Materials and Methods. (a) Normal replicative intermediates of the rolling circle type (double-stranded circle with single-stranded tail); (b) linear single-stranded DNA of unit length cleaved from the rolling circle by fd gene 2 protein; (c) a single-stranded circle cleaved and circularized by fd gene 2 protein; (d) a double-stranded circle with a double-stranded tail which was presumably generated by strand switching of T7 DNA polymerase; (e) linear single-stranded DNA of more than unit length; (f) a partially double-stranded circle. The transition of single-stranded and double-stranded regions are marked by an arrow

DISCUSSION

An intrinsic question of DNA synthesis of small phages is how the replicating viral strand becomes circularized.

Phage ϕX174 needs gene A protein for cleaving the phage RF I. The 5′ end of the growing viral strand is always looped back to the replication fork [17]. The attachment of gene A protein to the 5′ terminus was thought to support unwinding of the double strand and to keep the strand close to the 3′ end when the DNA is cleaved after a round of viral strand synthesis. The energy from RF cleavage is presumably conserved in the linkage between gene A protein and DNA for circularization of the adjacent single-stranded DNA ends.

In this paper we show that the enzyme responsible for the formation of circular single strands of phage fd is the phage-encoded gene 2 protein. The T7 enzymes are only required

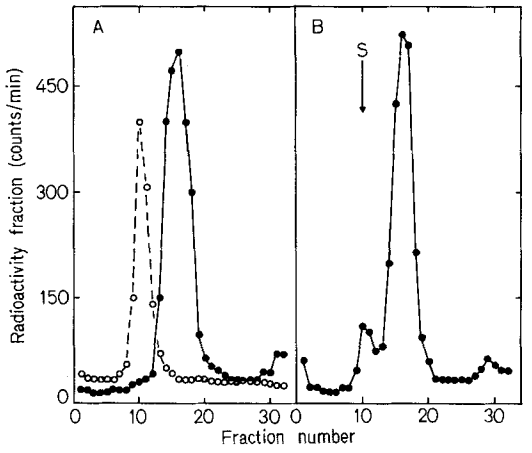

Fig. 7. *Replication of nicked fd RF with T7 gene 4 protein and T7 DNA polymerase* (A) In the absence of the T7 enzymes unlabeled phage fd RF I (100 ng) was completely cleaved with 0.32 unit of fd gene 2 protein, and the protein was inactivated by heat. Incubation and analysis was as in Fig. 2 with the ^{3}H label in dTTP (●——●; 700 counts min^{-1} pmol^{-1}). Further addition of gene 2 protein was omitted in this experiment. Internal marker was circular single-stranded ^{14}C-labeled fd DNA (O– –O; 1110 counts/min). (B) The incubation and analysis were performed as for A, but additional fd gene 2 protein (0.16 unit) was added together with the T7 enzymes. The position of circular single-stranded fd DNA is marked by the arrow S

to drive the replication of the fd RF. The topoisomerase activity of gene 2 protein, which is assumed to be involved in circularization, is independent of ATP [4]. Furthermore, the enzyme cannot conserve energy like ϕX gene A protein, because gene 2 protein does not bind tightly to the cleaved DNA [4]. We therefore suggest that the energy for circularization is derived from the cleavage of the replicating viral single strand. The enzyme might attach to the single strand which has been cleaved off and catalyze joining of the 3′ end to the 5′ end. Differences between fd gene 2 protein and ϕX174 gene A protein in replication are also visible in the electron microscope. Phage fd replication intermediates bear extended tails which are not looped back to the replication fork (I. Bäumel et al., unpublished). The different properties of the two enzymes have been discussed elsewhere [16].

As shown in Fig. 7, T7 gene 4 protein and T7 DNA polymerase can use a nicked fd RF as substrate for DNA synthesis. No free single strands can be seen in the absence of fd gene 2 protein (Fig. 7A). They are readily observed when gene 2 protein is added together with the T7 enzymes for replication of specifically nicked fd RF (Fig. 7B). Although gene 2 protein is necessary in an *Escherichia coli* system to start unwinding by *rep* protein at a specific nick (K. Geider, I. Bäumel, and T. F. Meyer, unpublished results), the T7 enzymes can apparently do the unwinding without a helper protein.

Data from sedimentation, gel electrophoresis and from the electron microscope show the formation of circular fd single strands. Like cleavage of the viral strand from a rolling circle, circularization also depends on the function of fd gene 2 protein. This enzyme is neither active on fd single strands nor on relaxed RF DNA [4]. A replicating intermediate is therefore necessary to activate the recognition sequence on the fd viral strand for cleavage by gene 2 protein. Quantitative evaluation of the data from the three methods show that about 5% of the template were detected as single-stranded circles. Priming of these circles and synthesis of complementary DNA as observed in the electron microscope may diminish the actual number of single-stranded circles, which might well be up to 10% of the RF molecules replicated by the three enzymes.

The T7 enzymes for replication of fd RF are a simple system which consists of relatively stable components. They replicate the DNA for at least two rounds of viral strand synthesis. Circularization of viral strands by gene 2 protein is efficient as the single-stranded circles outnumber the linear single strands. As discussed for a replication system with phage T4 enzymes [18], rolling circles with single-stranded tails of unit length might reflect a lag period for gene 2 protein in the cleavage reaction.

A part of the replicative intermediates contains double-stranded tails, which arise from strand switching of T7 DNA polymerase. This reaction limits the use of the T7 enzymes for synthesis of fd viral strands. These limitations are less prominent for replication of fd RF with phage T4 enzymes [18], although replicating viral strands are barely circularized in that system by fd gene 2 protein in contrast to replication with the T7 enzymes.

REFERENCES

1. Lin, N. S.-C. & Pratt, D. (1972) *J. Mol. Biol. 72*, 37–49.
2. Fidanián, H. M. & Ray, D. S. (1972) *J. Mol. Biol. 72*, 51–63.
3. Meyer, T. F. & Geider, K. (1979) *J. Biol. Chem. 254*, 12636–12641.
4. Meyer, T. F. & Geider, K. (1979) *J. Biol. Chem. 254*, 12642–12646.
5. Meyer, T. F., Geider, K., Kurz, C. & Schaller, H. (1979) *Nature (Lond.) 278*, 365–367.
6. Geider, K. & Meyer, T. F. (1978) *Cold Spring Harbor Symp. Quant. Biol. 43*, 59–62.
7. Kolodner, R. & Richardson, C. C. (1978) *J. Biol. Chem. 253*, 574–584.
8. Scherzinger, E., Lanka, E., Morelli, G., Seiffert, D. & Yuki, A. (1977) *Eur. J. Biochem. 72*, 543–558.
9. Romano, L. J. & Richardson, C. C. (1979) *J. Biol. Chem. 254*, 10476–10482.
10. Grippo, P. & Richardson, C. C. (1971) *J. Biol. Chem. 246*, 6867–6873.
11. Geider, K. & Kornberg, A. (1974) *J. Biol. Chem. 249*, 3999–4005.
12. Geider, K., Lechner, H. & Hoffmann-Berling, H. (1972) *J. Mol. Biol. 69*, 333–348.
13. Davis, R., Simon, M. & Davidson, N. (1971) *Methods Enzymol. 21D*, 413–428.
14. Richardson, C. C., Romano, L. J., Kolodner, R., LeClerc, J. E., Tamanoi, F., Engler, M. J., Dean, F. B. & Richardson, D. S. (1978) *Cold Spring Harbor Symp. Quant. Biol. 43*, 427–440.
15. Brutlag, D., Schekman, R. & Kornberg, A. (1971) *Proc. Natl Acad. Sci. USA, 68*, 2826–2829.
16. Meyer, T. F. & Geider, K. (1981) in *Mechanistic Studies of DNA Replication and Genetic Recombination*, ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XIX (Alberts, B. & Fox, C. F., eds) pp. 579–588, Academic Press, New York.
17. Eisenberg, S., Griffith, J. & Kornberg, A. (1977) *Proc. Natl Acad. Sci. USA, 74*, 3198–3202.
18. Meyer, T. F., Bäumel, I., Geider, K. & Bedinger, P. (1981) *J. Biol. Chem. 256*, 5810–5813.

G. Harth, I. Bäumel, T. F. Meyer, and K. Geider, Abteilung für Molekulare Biologie, Max-Planck-Institut für Medizinische Forschung, Jahnstraße 29, D-6900 Heidelberg, Federal Republic of Germany