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Replication of Bacteriophage M13

XIV. Differential Inhibition of the Replication of M13 and M13 Miniphage in a Mutant of *Escherichia coli* Defective in the 5′ → 3′ Exonuclease Associated with DNA Polymerase I

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Previous studies have shown that M13 single-strand synthesis is inhibited at nonpermissive temperature in *Escherichia coli polA*ex1, a temperature-sensitive mutant defective in the 5′ → 3′ exonuclease activity of polymerase I (T.-C. Chen and D. S. Ray, J. Mol. Biol. **106**:589-604, 1976). Under these conditions the formation of covalently closed replicative form (RF) molecules is greatly reduced, and miniature forms of RF accumulate. We show here that the accumulation of mini-RFs is the consequence of a differential inhibition of the replication of unit-length phage and preexisting miniphage rather than a de novo production of miniphage. Mini-RFs do not accumulate even after as many as nine cycles of growth in the mutant host infected only with unit-length phage. Mixed infections of the mutant host with plaque-purified unit-length phage and a single cloned miniphage show that discontinuities in the mini-RFs are joined with higher efficiency than are those contained in unit-length RFs. After a shift to nonpermissive temperature during single-strand synthesis in cells infected with plaque-purified phage alone, M13 RFs are found largely as RFII molecules (RF form having one or more single-strand discontinuities) containing only a single discontinuity in the viral strand. The inability of the accumulated unit-length RFII molecules to actively replicate may reflect the presence of either a bound protein or RNA primer on the 5′ terminus of the viral strand and provides further support for the existence of distinct initiation and termination events in the synthesis of the viral strand.

We have previously studied the replication of filamentous bacteriophage M13 in the *Escherichia coli polA*ex1 mutant (1). This mutant, originally isolated and characterized by Konrad and Lehman (10), contains a temperature-sensitive 5′ → 3′ exonuclease associated with DNA polymerase I (15). During replicative-form (RF) → single-strand (SS) DNA synthesis, this strain is defective in making M13 supercoiled DNA and accumulates mini-RF molecules ranging in size from 0.2 to 0.5 times genome length. Earlier stages of M13 replication have also been found to be inhibited in this mutant (2).

Since filamentous miniphage are known to have duplications of a portion of the origin of replication (4; T.-C. Chen and D. S. Ray, submitted for publication, they could perhaps be enriched in the mutant host as a consequence of their replication advantage. Experiments presented here show that mini-RFs do not accumulate in the mutant host when infected with phage that have been repeatedly plaque purified to eliminate contaminating miniphage. Furthermore, by using a phage preparation known to contain miniphage, we have shown that the accumulation of mini-RF molecules in the *E. coli polA*ex1 strain is caused by a differential inhibition of the replication of unit-length M13 relative to that of preexisting miniphage.

MATERIALS AND METHODS

Phage. M13JG was kindly provided by Jack Griffith and has been described previously (9). Approximately 30% of the phage in this preparation were found to be miniphage. Bacterial strains, media, and centrifugation techniques as well as labeling of phage-infected cells have also been described earlier (1).

Growth and isolation of phage. A 20-ml culture of *E. coli polA*ex1 (or its revertant) was grown at 30°C to a density of 2×10^8 cells per ml. M13JG was added at a multiplicity of infection (MOI) of 50. At 30 min after infection the culture was either left at 30°C or transferred to 38°C. [^{3}H]thymidine was then added at a concentration of 10 μCi/ml. After a labeling period of 3 h, the culture was cooled on ice, and the cells were

removed by centrifuging for 2 min at 12,000 rpm in a Sorvall SS34 rotor at 0°C. The supernatant was made 0.5 M in NaCl and 5% in polyethylene glycol (PEG 6000). After 30 min on ice, the phage were harvested by centrifugation for 5 min at 15,000 rpm in a Sorvall SS34 rotor at 0°C. The phage pellet was resuspended in 5 ml of sterile 0.01 M Tris–0.001 M EDTA (pH 8). A second polyethylene glycol precipitation was carried out in the presence of 0.5% Sarkosyl to remove contaminating *E. coli* pili. The resultant phage pellet was resuspended in 1 ml of sterile 0.01 M Tris–0.001 M EDTA (pH 8).

Gel electrophoresis. Electrophoresis was performed in gels set in glass tubes of 6-mm internal diameter and 20-cm length. Agarose disk gels were prepared as described by Suggs and Ray (13). Agarose was dissolved in electrophoresis buffer by autoclaving for 15 min (or, alternatively, irradiated for 1 min in a microwave oven) and poured into gel tubes sealed with Parafilm. After the gels hardened, the Parafilm was removed, the top few centimeters of gel were sliced off to provide a flat surface, and the bottoms of the gels were covered with cheesecloth.

For separation of M13 full-length phage and miniphage, 3% agarose disk gels were used with the electrophoresis buffer (9) containing 14 g of glycine and 3 g of Tris per liter (pH 8.9). Agarose gels (2%) were used to analyze DNA samples in an electrophoresis buffer (12) containing 0.036 M Tris, 00.3 M NaH_2PO_4, 1 mM EDTA, and 0.05% sodium dodecyl sulfate. In our experiments we often omit sodium dodecyl sulfate. After electrophoresis the gels were sliced into 1.8-mm disks and solubilized by autoclaving in 1 ml of distilled water prior to counting in a toluene–Triton X-100–2,5-diphenyloxazole (PPO) (2 liters, 1 liter, 8 g, respectively) scintillation fluid.

EtBr-CsCl equilibrium centrifugation. Equilibrium centrifugation in the presence of ethidium bromide (EtBr) was performed in the following manner. A 2.9-ml sample of DNA, 0.1 ml of EtBr at 4 mg/ml, and 2.8 g of CsCl were mixed together and centrifuged for 50 h at 34,000 rpm 17°C in a Beckman type 40.2 rotor. Fractions of 6 drops were collected from the bottom of the tube by pumping oil in from the top.

RESULTS

Lack of mini-RF molecules in *E. coli polA*ex1 infected by plaque-purified M13. Our previous experiments on M13 replication in *E. coli polA*ex1 (1) utilized M13 phage that had been grown in a large-scale fermentor, a condition under which miniphage have been observed to be produced (4). In these experiments the *E. coli polA*ex1 strain was found to be defective in making M13 supercoiled DNA as indicated by an excessive accumulation of material sedimenting at the rate of RFII. In addition, a component sedimenting slower than unit-length RFII was observed. This slow-sedimenting component was found to consist of both open and closed circular forms of mini-RF molecules.

We have reexamined M13 RF → SS replication in *E. coli polA*ex1 cells using a plaque-purified M13 preparation. Cells grown and infected at permissive temperature were shifted at 1 h after infection to 38°C, a temperature at which progeny SS synthesis is only partially inhibited but at which accumulation of mini-RF molecules was previously found to be most pronounced (1). Five minutes after the temperature shift, tritiated thymidine was added to label-replicating DNA. Ten minutes after addition of the label, the cells were harvested, lysed, and sedimented through a neutral high-salt-sucrose gradient. None of the incorporated label sedimented slower than unit-length RFII, the region of the gradient where mini-RF molecules have been shown to sediment (Fig. 1a). In agreement with our previous results, the defective 5′ → 3′ exonuclease function associated with DNA polymerase I is required for sealing RFII molecules into RFI molecules (RF molecules having both strands covalently closed) as evidenced by the excessive accumulation of material sedimenting at the rate of RFII in Fig. 1a. At this temperature (1), some viral SS can still be synthesized, and these are observed here in fractions 32-37. Alkaline sedimentation of this material shows that the labeled viral strands are almost entirely unit-length linear molecules and indicates that the accumulated RFII molecules contained only a single discontinuity in the viral strand (Fig. 1b) and are uncontaminated by mini-length molecules. Furthermore, the plaque-purified phage were serially passed nine times at a high MOI (MOI = 100) on *E. coli polA*ex1. When such a phage stock was used for infection, the resulting radioactivity profile resembled that of Fig. 1a. The absence of mini-RFs even after repeated cycles of growth on the *polA*ex1 host indicates that mini-RFs are not produced de novo at abnormally high frequency in this mutant.

Preferential replication of preexisting miniphage in *E. coli polA*ex1 cells. Since the *polA*ex1 mutation does not result in the generation of mini-RF molecules during M13 replication, the accumlation of mini-RF molecules in mutant cells infected with M13 phage grown through several cycles without plaque purification could possibly result from a preferential replication of preexisting miniphage contained in the original phage preparation. To test this possibility, we have used a phage stock, designated M13JG, which is known to contain miniphage (8). M13JG phage were grown in either the *E. coli polA*ex1 mutant or its revertant for 3.5 h at 30 or 38°C in the presence of tritiated thymidine. The phage were then harvested, concentrated by polyethylene glycol precipitation, and analyzed on 3% agarose gels.

Figure 2 shows the resulting radioactivity profile after gel electrophoresis. In these gels, miniphage particles migrated ahead (fractions 12 to 14) of unit-length phage particles (fractions 6 to

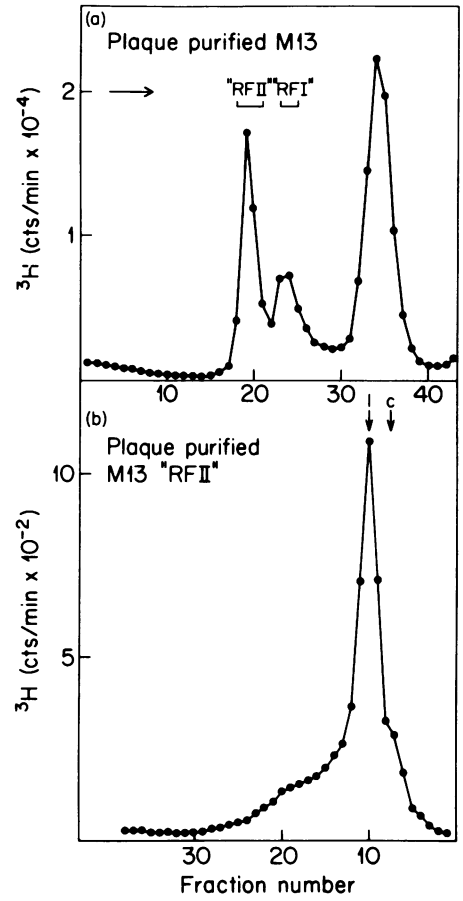

FIG. 1. *Velocity sedimentation analysis of M13 RF → SS synthesis in E. coli polAex1 cells infected by plaque-purified M13. An E. coli polAex1 culture was grown at 30°C to a density of 2 × 10^8 cells per ml and was infected with plaque-purified M13 at an MOI of 100. At 60 min after infection the culture was shifted to 38°C. At 5 min after the temperature shift [^{3}H]-thymidine was added to the culture at a concentration of 20 μCi/ml for 10 min. Cells were then harvested, lysed, and sedimented through a 34-ml, 5 to 20% sucrose gradient for 17 h at 24,000 rpm in a SW27 rotor at 5°C. DNA sedimenting at the rate of M13 RFII (fractions 18 to 21) was pooled, dialyzed, and further sedimented through a 3.5-ml, 5 to 20% alkaline sucrose gradient containing 0.2 N NaOH and 5mM EDTA. ^{32}P-labeled M13 SS were included as markers for unit-length circles (c) and linears (l). Centrifugation was for 4 h at 56,000 rpm in a SW60 rotor at 20°C. The direction of sedimentation is indicated by the horizontal arrow. (a) Cell lysate analyzed by neutral velocity sedimentation; fractions were collected from the top of the tube. (b) RFII DNA analyzed by alkaline velocity sedimentation; fractions were collected from the bottom of the tube.*

8). Of the total labeled phage made in the revertant at 30°C, 21.5% of the radioactivity was in miniphage particles and 78.5% in unit-length phage particles (Table 1). In the *E. coli polA*ex1 mutant at the same temperature, 39.7% of the label was contained in miniphage and 60.3% in unit-length phage. The proportion of miniphage was increased further at 38°C. At this temperature the distribution of radioactivity between miniphage particles and unit-length phage particles in the revertant was similar to that at 30°C. On the other hand, nearly 57% of the total tritium label resided in miniphage particles in the *E. coli polA*ex1 mutant at 38°C. These results indicate a preferential inhibition of replication of M13 unit-length phage relative to the miniphage in the *E. coli polA*ex1 mutant.

Preferential closure of mini-RFII. The M13JG phage preparation and other prepara-

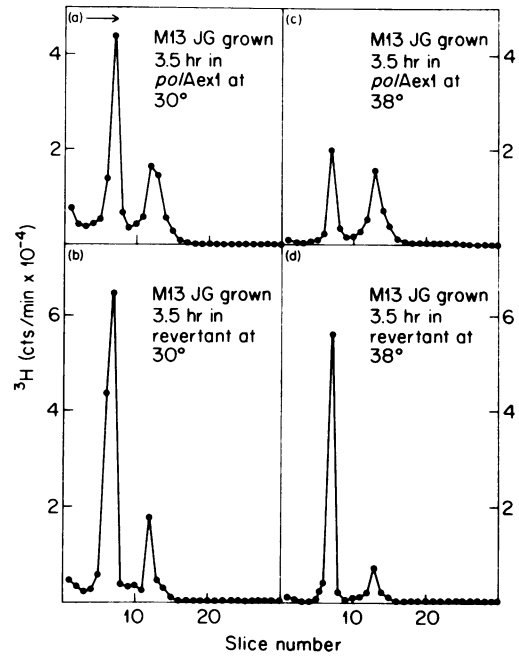

FIG. 2. *Gel electrophoresis of M13 phage and miniphage grown in E. coli polAex1 and its methyl methane sulfonate-resistant revertant. E. coli polAex1 cells (or its methyl methane sulfonate-resistant revertant cells) were grown to a cell density of 2 × 10^8 cells per ml at 30°C and were infected with M13JG at an MOI of 50. After 30 min the culture was divided into two equal portions. One was left at 30°C and the other one was transferred to 38°C. [^{3}H]thymidine was added to each culture to a final concentration of 10 μCi/ml and 2 μg/ml, and incubation was continued for another 3 h. At the end of labeling, cells were removed by low-speed centrifugation. Phage were harvested and purified from the supernatant by two serial polyethylene glycol precipitations. Samples were then analyzed on 3% agarose tube gels in Tris-glycine buffer (pH 8.9). The direction of migration is indicated by the arrow. (a) M13JG grown in E. coli polAex1 at 30°C; (b) M13JG grown in the revertant at 30°C; (c) M13JG grown in E. coli polAex1 at 38°C; (d) M13JG grown in the revertant at 38°C.*

TABLE 1. *Relative yields of M13 and M13 miniphage in E. coli polAex1 and a temperature-resistant revertant*

M13JG growth in	Growth temp (°C)	Relative yields (%)	
		Unit-length phage	Miniphage
*E. coli polA*ex1	30	60.3	39.7
*E. coli polA*ex1	38	43.3	56.7
Revertant	30	78.5	21.5
Revertant	38	80.2	19.8

tions grown in bulk contain a heterogeneous population of miniphage. To investigate the replication of both unit-length and mini-RF molecules in the mutant host, we have cloned miniphage by the methods described by Hewitt (9) and have obtained phage preparations containing unit-length phage and miniphage of only a single homogeneous length. One such miniphage, JC04, has been used here to investigate the replication of mini-RFs. This particular miniphage is 35% of unit length and contains a duplication of a portion of the replication origin (Chen and Ray, submitted for publication).

*E. coli polA*ex1 cells were infected by either plaque-purified M13 alone or cloned miniphage JC04 in the presence of M13 helper phage. In the latter case, there were 2.5 times as many M13 helper phage as there were JC04 miniphage. The infected cells were labeled with tritiated thymidine for 10 min during late infection, lysed, and sedimented through neutral high-salt-sucrose gradients. These gradients were allowed to run long enough that all the unit-length M13 SS pelleted at the bottom and the mini-RFII and mini-RFI molecules were well separated from unit-length RFII and RFI molecules.

Figure 3b displays such a radioactivity profile in which cloned miniphage JC04 was used. Labeled DNA at fractions 18 to 21 in Fig. 3b was identified as mini-RFII DNA based on its migration as a single homogeneous peak faster than unit-length RFII (Fig. 4a) and its banding at the same density as unit-length RFII in an EtBr-CsCl gradient (Fig. 5a). The small amount of material banding at a density greater than that of M13 supercoiled RFI in Fig. 5a possibly reflects the presence of a small amount of relaxed but covalently closed RFs. Labeled DNA at fractions 22 to 25 in Fig. 3b was characterized as mini-RFI DNA based on its migration as a single homogeneous peak faster than unit-length RFII and mini-RFII molecules (Fig. 4b) and its banding largely as supercoiled DNA in an EtBr-CsCl gradient (Fig. 5b). The small amount of material banding at the density of RFII results from an overlap of the mini-RFII peak in the original sucrose gradient and possibly from a small amount of random nicking during handling. In addition, upon digestion with various restriction endonucleases, DNA from fractions 18 to 21 and 22 to 25 both gave rise to the same spectrum of restriction fragments. Thus, we conclude that these are open and closed forms of the same duplex DNA.

Comparison of Fig. 3a and 3b shows an unexpected difference in the replication of unit-length and mini-RFs. The efficiency of closure of RFII molecules to form supercoiled RFI molecules is clearly much higher for the mini-RFs than for unit-length RFs. Since JC04 has been found to contain only a single functional viral strand origin (Chen and Ray, submitted for pub-

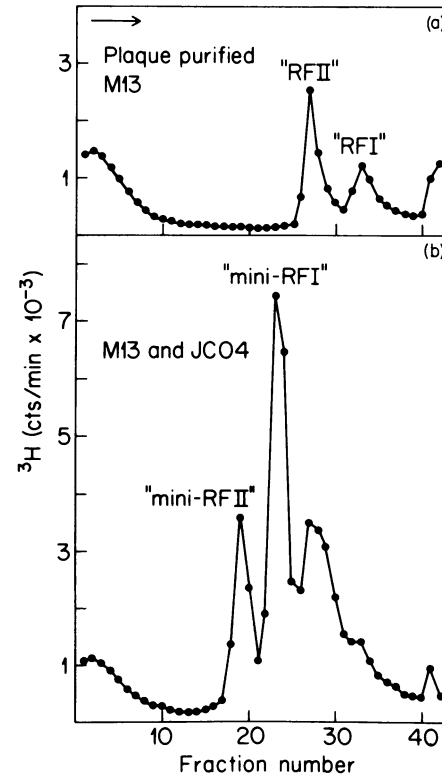

FIG. 3. *Neutral velocity sedimentation analysis of M13 RF → SS in E. coli polAex1 cells infected by (a) plaque-purified M13 or (b) M13 and cloned miniphage JC04. Cells were grown to a density of 2 × 10^8 cells per ml at 30°C and were infected with plaque-purified M13 alone (MOI = 50) or with M13 (MOI = 50) and JC04 (MOI = 20) together. At 60 min after infection cultures were transferred to 38°C. After 5 min, [^{3}H]thymidine was added to each culture at a concentration of 20 μCi/ml. After 10 min of labeling, cells were harvested, lysed, and sedimented through 5 to 20% neutral high-salt-sucrose gradients. Centrifugation was for 24 h at 24,000 rpm in a SW27 rotor at 5°C. The direction of sedimentation is indicated by the horizontal arrow.*

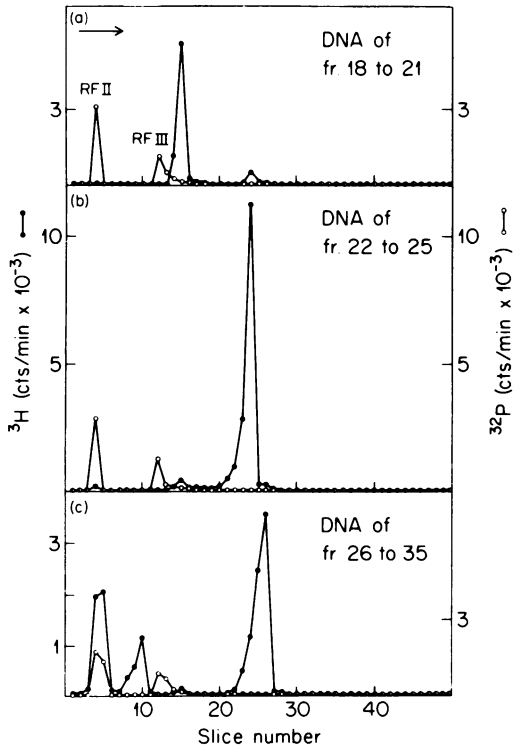

FIG. 4. *Gel electrophoretic analysis of pooled DNA fractions from E. coli polAex1 cells coinfected with M13 and cloned miniphage JC04. (a) DNA from fractions 18 to 21 in Fig. 3b; (b) DNA from fractions 22 to 25 in Fig. 3b; (c) DNA from fractions 26 to 35 in Fig. 3b. DNA from various regions in Fig. 3b were pooled, dialyzed, and concentrated by ethanol precipitation. Samples were mixed with ^{32}P-labeled M13 RFII and RFIII molecules and subjected to gel electrophoresis in 2% agarose gels in Tris-phosphate-EDTA buffer. Electrophoresis was for 2 h at 160 V.*

lication) there was no a priori reason to expect any difference in the closure of mini-RFII molecules formed during asymmetric viral strand synthesis.

To determine also the ratio of unit-length RFII to RFI in cells coinfected with miniphage, DNA fractions were pooled from the region (fractions 26 to 35 in Fig. 3b) where unit-length RFII and RFI sediment, dialyzed, concentrated by ethanol precipitation, and analyzed by gel electrophoresis. This DNA migrated as three discrete peaks (Fig. 4c). The slowest-migrating peak (slices 6 and 7) is unit-length RFII since it comigrated with ^{32}P-labeled M13 RFII marker. The middle peak (slices 9 and 10) migrated at the position of unit-length RFI just behind a unit-length RFIII (linear RF) marker. In other experiments (not shown) this material comigrated with ^{32}P-labeled M13 RFI. The third peak (slices 26 and 27) has been identified as mini-SS based on its density in neutral CsCl gradients and its electrophoretic mobility relative to M13 SS. Thus, the ratio of unit-length RFII to RFI made in the presence of miniphage is very similar to that made in the absence of miniphage (Fig. 3a). These results indicate that the mini-RFII molecules become convalently closed with much higher efficiency than unit-length RFII in the *E. coli polA*ex1 mutant.

DISCUSSION

Filamentous miniphage have been discovered that are unable to express any *trans*-acting gene function and are only propagated upon coinfection with a full-length helper (4, 5, 8). The generation of miniphage during replication is not a normal event since discrete plaques produced by

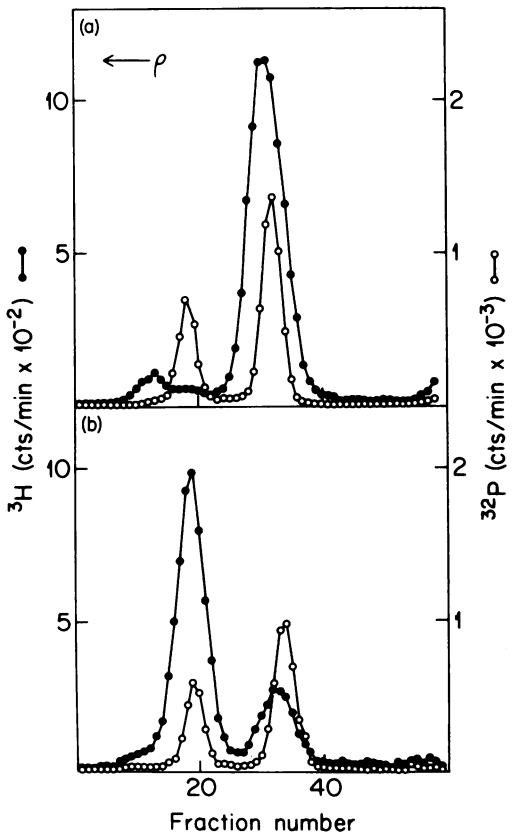

FIG. 5. *Equilibrium centrifugation of mini-RFs in EtBr-CsCl gradients. JC04 RFII and RFI DNA were each pooled, dialyzed, and ethanol precipitated from a sucrose gradient which had been centrifuged for 17 h instead of 24 h as in Fig. 3. Samples were mixed with ^{32}P-M13 RFs and subjected to equilibrium centrifugation in EtBr-CsCl gradients. Centrifugation was for 50 h at 34,000 rpm in a Beckman type 40.2 rotor at 17°C. Buoyant density increases from right to left. (a) JC04 RFII; (b) JC04 RFI.*

a stock containing miniphage yield phage which are all full-length particles (9). However, once a miniphage is produced, it replicates readily and tends to outgrow the full-length phage in a wild-type host.

We have shown that the accumulation of mini-RF molecules in the *E. coli polA*ex1 strain is not due to a de novo production of mini-RFs as a direct consequence of the *polA*ex1 mutation, but rather that this mutant host replicates preexisting miniphage better than it replicated unit-length M13. The *E. coli polA*ex1 strain is defective in making unit-length M13 supercoiled DNA and therefore accumulates M13 RFII molecules. In contrast, mini-RFII molecules are converted efficiently into mini-RFI DNA even though unit-length RF molecules in the same cells remain largely in an open circular form. The more efficient replication of miniphage thus may be a direct result of the preferential closure of mini-RFII molecules. It should be noted that both the miniphage used here and those isolated in Zinder's laboratory have one or more duplications of the region containing the origin of replication (4; Chen and Ray, submitted for publication) which might affect, in an unknown way, the closure of the RFII.

Our previous results (1), as well as results presented here, indicate a reduced ability of the unit-length RFII molecules accumulated in the *polA*ex1 mutant to actively replicate. This result is consistent with the existence of distinct termination and initiation steps in viral strand synthesis. A pause in viral strand synthesis at the origin of replication had already been indicated by the finding that the discontinuity in wild-type RFII molecules is located at the viral strand origin of replication (13).

In the rolling circle model, as conceived by Gilbert and Dressler (7), covalent closure of the discontinuity in the viral strand after a round of replication is not a necessary step in the further replication of the viral strand. In such a continuous rolling circle model, replication might even be stimulated by preventing the closure of RFII molecules. The unexpected inhibition of viral strand synthesis in the absence of the polymerase I 5′ → 3′ exonuclease activity (1) suggests that each round of viral strand synthesis is terminated by the formation of RFI before the next round can be initiated. One step in the termination of a round of viral strand synthesis appears to involve degradation of the 5′ terminus of the viral strand. Possible termination events might include the removal of either a protein or an RNA primer from the 5′ terminus by the concerted action of the 5′ → 3′ exonuclease and the polymerase activities of DNA polymerase I and subsequent closure of the viral strand by ligase. Before initiation of another round of DNA replication, the final step in the termination of viral strand synthesis appears to be the conversion of RFIV, the relaxed, covalently closed RF molecule, to the supercoiled form, RFI, by the host gyrase (K. Horiuchi, personal communication). Upon conversion to RFI, a subsequent round of synthesis can be initiated by gene 2-mediated nicking of the viral strand at the origin (6).

We had previously suggested that M13 viral strands might be synthesized in a discontinuous manner (1). However, results presented here with plaque-purified M13 phage preparations do not support this suggestion. M13 RFII molecules accumulated in the mutant host contain linear viral strands of unit length. Viral strand fragments of less than unit length observed in our earlier work can now be accounted for by the miniature species of RF known to be present in those preparations (1). Similarly, the observation by DasGupta and Mitra (3) that nascent replicative forms of M13 isolated from the *polA*ex1 mutant contain multiple discontinuities might be explained by the presence of mini-RF molecules and also by the possible excision-repair of uracil residues in the nascent DNA strands (14).

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LITERATURE CITED

1. **Chen, T.-C. and D. S. Ray.** 1976. M13 replication in a mutant of *Escherichia coli* defective in the 5′ → 3′ exonuclease associated with DNA polymerase I. J. Mol. Biol. **106**:589-604.
2. **DasGupta, S., and S. Mitra.** 1977. The role of DNA polymerase I-associated 5′-exonuclease in replication of coliphage M13 replicative-form DNA. Biochem. Biophys. Res. Commun. **78**:1108-1113.
3. **DasGupta, S., and S. Mitra.** 1978. Structure of nascent replicative form DNA of coliphage M13. Proc. Natl. Acad. Sci. U.S.A. **75**:153-157.
4. **Enea, V., K. Horiuchi, B. G. Turgeon, and N. D. Zinder.** 1977. Physical map of defective interfering particles of bacteriophage fl. J. Mol. Biol. **111**:395-414.
5. **Enea, V., and N. D. Zinder.** 1975. A deletion mutant of bacteriophage fl containing no intact cistrons. Virology **68**:105-114.
6. **Fidanián, H. F., and D. S. Ray.** 1972. Replication of bacteriophage M13. VII. Requirement of the gene 2 protein for the accumulation of a specific RFII species. J. Mol. Biol. **72**:51-63.
7. **Gilbert, W., and D. Dressler.** 1968. DNA replication: the rolling circle model. Cold Spring Harbor Symp. Quant. Biol. **32**:473-484.
8. **Griffith, J., and A. Kornberg.** 1974. Mini M13 bacteriophage: circular fragments of M13 DNA are replicated

and packaged during normal infections. Virology **59**:139–152.

9. **Hewitt, J. A.** 1975. Miniphage—a class of satellite phage to M13. J. Gen. Virol. **26**:87–94.
10. **Konrad, E. B., and I. R. Lehman.** 1974. A conditional lethal mutant of *Escherichia coli* K12 defective in the 5′ → 3′ exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. U.S.A. **71**:2048–2051.
11. **Lica, L., and D. S. Ray.** 1977. *In vivo* cross-linking of a phage-specific DNA binding protein to the single-stranded DNA of bacteriophage M13 by ultraviolet irradiation. J. Mol. Biol. **115**:45–49.
12. **Loening, J. E.** 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis. Biochem. J. **113**:131–138.
13. **Suggs, S. V., and D. S. Ray.** 1977. Localization of the origin for M13 single strand synthesis. J. Mol. Biol. **110**:147–163.
14. **Tye, B.-K., P.-O, Nyman, I. R. Lehman, S. Hochhauser, and B. Weiss.** 1977. Transient accumulation of Okazaki fragments as a result of uracil incorporation into nascent DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:154–157.
15. **Uyemura, D., D. C. Eichler, and I. R. Lehman.** 1976. Biochemical characterization of mutant forms of DNA polymerase I from *Escherichia coli*. II. The *polA*ex1 mutation. J. Biol. Chem. **251**:4085–4089.