JOURNAL OF VIROLOGY, Apr. 1977, p. 23-30
Vol. 22, No. 1

Printed in U.S.A.

Inhibition of Bacteriophage M13 and φX174 Duplex DNA Replication and Single-Strand Synthesis in Temperature-Sensitive *dnaZ* Mutants of *Escherichia coli*

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Received for publication 8 October 1976

A functional *dnaZ* product, known to be essential for host DNA polymerization and for the synthesis of M13 and φX174 parental replicative-form (RF) DNA, is required also for RF replication and single-strand synthesis by both of these phages. All three stages of M13 and φX174 DNA replication (parental RF formation, RF replication, and single-strand synthesis) are inhibited in *dnaZ*ts mutants at elevated temperatures. In addition, the thermolabile step in M13 parental RF formation appears to occur after RNA priming; i.e., the synthesis of M13 RF DNA proceeded when a *dnaZ*ts mutant, infected at a nonpermissive temperature, was transferred to a permissive temperature in the presence of rifampin.

The single-strand (SS) DNA phages M13 and φX174 require host DNA replicative factors for the synthesis of their replicative intermediate and progeny viral DNAs (13, 17). Several *Escherichia coli* mutants, with defects in host chromosome replication, are deficient in gene products that are required by these phages in one or more stages of their DNA replication cycle: parental replicative-form (RF) synthesis, RF replication, or the asymmetric synthesis of progeny viral strands (for a summary, see 7).

We have previously presented evidence that the product of the DNA synthesis gene, *dnaZ*, is needed in vivo for the first stage of M13 and φX174 replication, the conversion of infecting viral SS DNA into duplex parental RF DNA (10). Wickner and Hurwitz (21) reported that the *dnaZ* gene product is required in vitro for DNA polymerase III activity on primed SS M13, φX174, and ST-1 templates. The observation that the *dnaZ* function is required for DNA polymerase III-catalyzed DNA synthesis in vitro suggests that the *dnaZ* product may participate in vivo in all stages of M13 and φX174 replication that have been shown to be effected by DNA polymerase III, i.e., RF replication and SS synthesis (3, 8, 18) (in addition to parental RF formation).

In this communication, we report that the replication of M13 and φX174 RF DNA and the synthesis of progeny SS DNA are inhibited in *dnaZ*ts mutants at nonpermissive temperatures. In addition, the *dnaZ* function in M13 parental RF formation in vivo appears to occur at a step after RNA priming. These data suggest a function for the *dnaZ* protein in the elongation phase of in vivo DNA synthesis or in an initiation step that occurs after the priming reaction.

MATERIALS AND METHODS

Bacterial and phage strains. K-12 strain AX727 *dnaZ*ts2016 and the φX174-sensitive K-12 strain GN727 *dnaZ*ts2016 were described previously (10). H727 is a *dnaZ*ts2016 recombinant between H5274 (obtained from D. Ray) and HAX727 (Hfr of AX727 constructed by F'*lac*$^+$ integration) and has the genotype K-12 F$^-$ *dnaZ*ts2016 *thy rpsE tsx*. C727 is a *dnaZ*ts2016 recombinant between *E. coli* C (obtained from R. McKee) and HAX727. It has the genotype C F$^-$ *dnaZ*ts2016 *rpsE tsx*. F'101/C600 was obtained from B. Bachmann, the male specific phage M13 from D. Ray, φX174h8ρ (retarded lysis) from C. Earhart, and φX174am3 (lysis defective) from R. L. Sinsheimer. AX727 TS$^+$, GN727 TS$^+$, H727 TS$^+$, and C727 TS$^+$ were spontaneous temperature-insensitive revertants of AX727, GN727, H727, and C727. F'*lac*$^+$ derivatives were obtained by mating with F'*lac*/RV (obtained from M. Malamy).

Media. Yeast extract-tryptone (YET) broth has been described (20). YET broth was supplemented with [^{3}H]thymidine at 10 μCi/0.12 μg per ml for radioactive labeling. Minimal phosphate medium was 0.8% nutrient broth (Difco), 1.5 mM NH_4OH, and 2.5 mM $MgSO_4$ (pH 7.0).

Preparation of phage stocks and marker DNAs. Nonradioactive M13 was prepared by infecting F'101/C600 (4×10^8 cells/ml) in YET broth with M13 at a multiplicity of infection of 50. After a 30-min adsorption period at 30°C, the culture was diluted 1:50 and grown in YET broth at 30°C to a cell density of 7×10^8 cells/ml. Cells were removed by low-speed centrifugation, and the phage was concentrated by

pelleting as described previously (10). ^{32}P-labeled M13 was grown similarly in minimal phosphate medium supplemented with 20 μCi of carrier free $^{32}PO_4^{3-}$ per ml. After concentration as above, the phages were purified on high-salt, 5 to 20% neutral sucrose gradients (10) and the viral DNA was isolated by cold phenol extraction (2).

ϕX174am3 and ϕX174h8ρ were grown in *E. coli* C in YET broth supplemented with 1.5 mg of $CaCl_2$ per ml. *E. coli* C cultures (2×10^8 cells/ml) were infected with either ϕX174am3 or ϕX174h8ρ at a multiplicity of infection of 3 and grown for 2.5 h at 37°C. The cultures were concentrated 25-fold into 0.1 M Tris-hydrochloride (pH 8.1) containing 0.5 mg of lysozyme per ml and 10 mM EDTA. After a 60-min incubation on ice, lysis was completed by shaking with a few drops of chloroform. To decrease viscosity and separate phage from debris, the lysate was drawn through a 0.5-inch (about 1.3-cm), 26-gauge needle. Debris was removed by low-speed centrifugation. Phage that were to be used as a source of marker DNA (ϕX174h8ρ) were gradient purified, and the DNA was extracted by the method of Brown and Dowell (2).

Analysis of M13 and ϕX174 DNA synthesis. Termination of isotope incorporation and lysis of M13-infected cells were accomplished as described by Ray et al. (15). ϕX174am3-infected cells were processed by the protocol of Dumas et al. (4), except that the lysate was incubated with Sarkosyl for 10 min at 37°C before Pronase treatment, rather than for 16 h at 0°C. Crude lysates of M13- and ϕX174am3-infected cells were analyzed on high-salt, 5 to 20% sucrose gradients (10). ^{32}P-labeled M13 DNA and unlabeled ϕX174h8ρ DNA were applied to the gradients as SS markers for M13- and ϕX174am3-infected cell lysates, respectively. The position of the ϕX174 marker was assessed by its biological activity in the spheroplast infectivity assay (9). Gradient fractions were collected from the bottom. Samples of 100 μl were spotted onto GF/A glass fiber filters, which, after drying, were washed with 3 ml of 5% trichloroacetic acid, dried, and counted.

RESULTS

Role of *dnaZ* in M13 parental RF formation. An active *dnaZ* product is required for the synthesis of both Ml3 and ϕX174 parental RF DNA (10). Inasmuch as different RNA priming mechanisms are used by each of these phages (16), it might be assumed that the *dnaZ* product would not be involved in the priming step of RF synthesis. This assumption could be tested in the M13 system. RNA priming on M13 infecting SS DNA is believed to be effected by rifampin-sensitive RNA polymerase (16). Therefore, if infection of a *dnaZ*ts mutant by M13 at a nonpermissive temperature rendered parental RF formation resistant to rifampin when the temperature subsequently was lowered, it may be concluded that the rifampin-sensitive step, i.e., priming, could be accomplished in the absence of an active *dnaZ* product. It was possible to do this experiment because the product of the *dnaZ*ts2016 allele regains activity immediately upon temperature reduction even in the absence of protein synthesis (Chu et al., in preparation). A culture of F'*lac*$^+$/AX727 was grown at 30°C and divided into three subcultures, all of which were then incubated at 41°C to inactivate the *dnaZ* product. Rifampin was added to two of these cultures either 5 min before or 5 min after infection by M13. As described in Fig. 1, the cultures were then transferred to 30°C and pulse-labeled with [^{3}H]thymidine. The third culture was infected and pulse-labeled at 41°C to demonstrate that no RF synthesis occurred at this temperature.

Infection of a *dnaZ*ts culture by M13 at 41°C resulted in a substantial amount of RF synthesis upon return of the culture to 30°C in the presence of rifampin (Fig. 1A). Cultures treated with rifampin before infection yielded no detectable peaks of phage DNA (Fig. 1B). There was no apparent RF synthesis in the culture infected and pulse-labeled at 41°C (Fig. 1C). We infer that RNA priming of infecting M13 single strands can occur in vivo in the absence of an active *dnaZ* product. The culture infected with M13 before rifampin treatment (Fig. 1A) contained two peaks with sedimentation properties consistent with RF I and RF II. The magnitude of the RF II peak (fraction 27) was unexpected. Formation of RF II DNA normally requires the gene 2 protein of M13 (5), and the synthesis of this protein should have been inhibited in a rifampin-treated culture. The amount of labeled material that sedimented as RF II varied from experiment to experiment; this variation might have been a result of the length of the 41°C preincubation period rather than an incomplete rifampin block on M13 gene 2 protein synthesis. Chloramphenicol, at concentrations that are adequate to prevent RF II formation in M13-infected cells (10), failed to eliminate RF II material from lysates of F'*lac*$^+$/AX727 cultures that were preincubated at 41°C and then transferred to 30°C (data not shown). It is possible that the material forming the RF II peak represented abnormal DNA synthesized in the presence of a partially reactivated *dnaZ* product or, alternatively, RF I breakdown products formed as a result of a nucleolytic activity present after the 41°C incubation.

Requirement for the *dnaZ* function in M13 and ϕX174 growth after parental RF formation. The effect of the *dnaZ* mutation of M13 and ϕX174 phage growth after parental RF formation was tested by shifting *dnaZ*ts and TS$^+$ cultures, which were infected and incu-

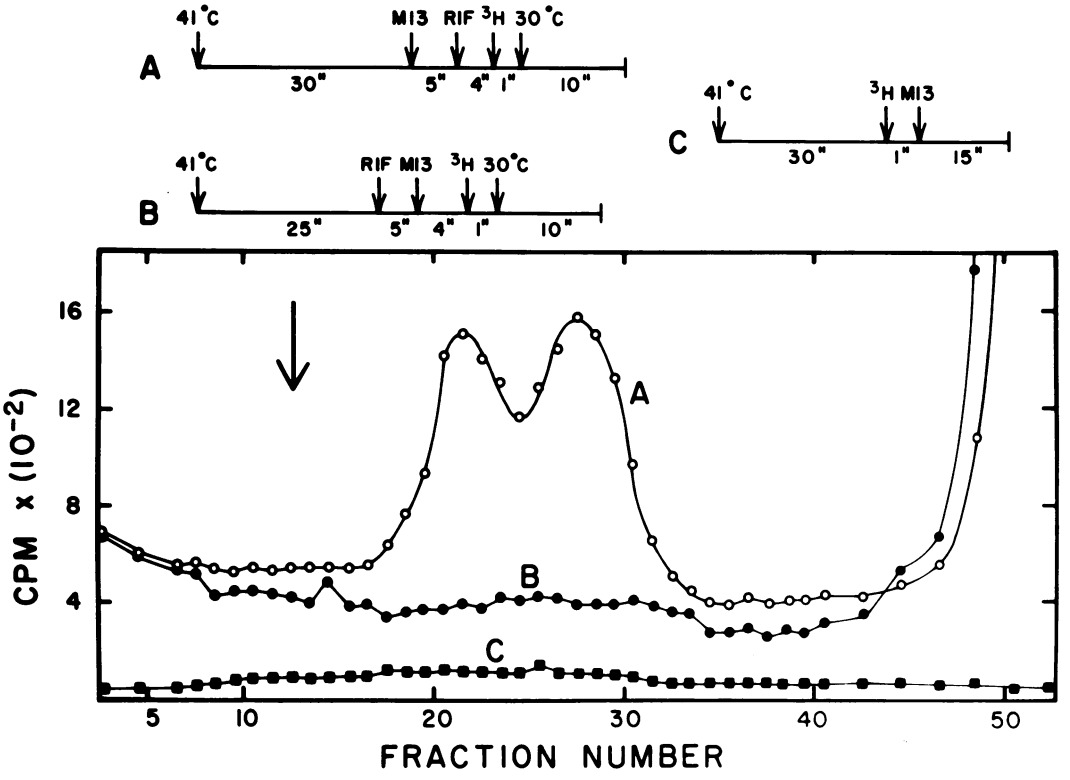

FIG. 1. *Formation of M13 parental RF at 41°C by a dnaZts culture in the presence of rifampin. F'lac⁺/AX727 dnaZts was grown to a density of 3.5 × 10⁸ cells/ml in YET broth at 30°C. Three samples were then transferred to 41°C. Culture A (○) was incubated for 30 min at 41°C and infected with M13 (multiplicity of infection = 100). Five minutes after infection, rifampin was added to a concentration of 200 μg/ml and the incubation of 41°C was continued for an additional 5 min. The culture was then pulse-labeled with [³H]thymidine for 10 min at 30°C. Culture B (●) was similarly treated; however, rifampin was added 5 min before infection by M13. Culture C (■) was not exposed to rifampin but was infected and pulse-labeled at 41°C. Cells were harvested, lysed, and sedimented through high-salt neutral sucrose gradients. Sedimentation in this and all other graphs was from right to left. The vertical arrow indicates the sedimentation position of [³²P]labeled M13 SS DNA.*

bated at a permissive temperature (to allow parental RF formation), to either 41 or 40°C (nonpermissive for DNA synthesis).

Cultures of F'*lac*⁺/H727 *dnaZ*ts and TS⁺ were grown and infected with M13 at 30°C. After 30 min, half of each culture was shifted to 41°C. Although phage production in the *dnaZ*ts and TS⁺ cultures was similar at 30°C, the *dnaZ*ts culture terminated phage synthesis after 30 min at 41°C (Fig. 2A). M13 growth was slowed in the revertant culture at 41°C; however, phage production continued throughout the course of the experiment. (The previously reported [20] immediate cessation of M13 phage production in another *dnaZ*ts strain [AX727] appears to result from an immediate halt of M13 SS synthesis, by temperature elevation, which probably is unrelated to the *dnaZ* mutation. M13 SS synthesis also was inhibited, although transiently, after a shift of an AX727 TS⁺ culture to 41°C [unpublished data].)

Shifting C727 *dnaZ*ts and TS⁺ cultures to 40°C after they were infected with φX174am3 and incubated at 35°C (selected as the permissive temperature) for 15 min inhibited phage growth only in the *dnaZ*ts culture (Fig. 2B). Although phage production in the *dnaZ*ts culture was detectable at 40°C for as long as 105 min, the rate of phage production, relative to that in the revertant culture, appeared to slow after 15 min at this temperature. Total phage yield in the *dnaZ*ts culture after 165 min at 40°C was approximately 1/100 the yield in the revertant culture. Phage growth in these cultures was comparable at 35°C. Inhibition of φX174 growth was previously observed by Taketo (19) in AX727 (*dnaZ*ts) spheroplasts that were transformed with φX174 RF DNA.

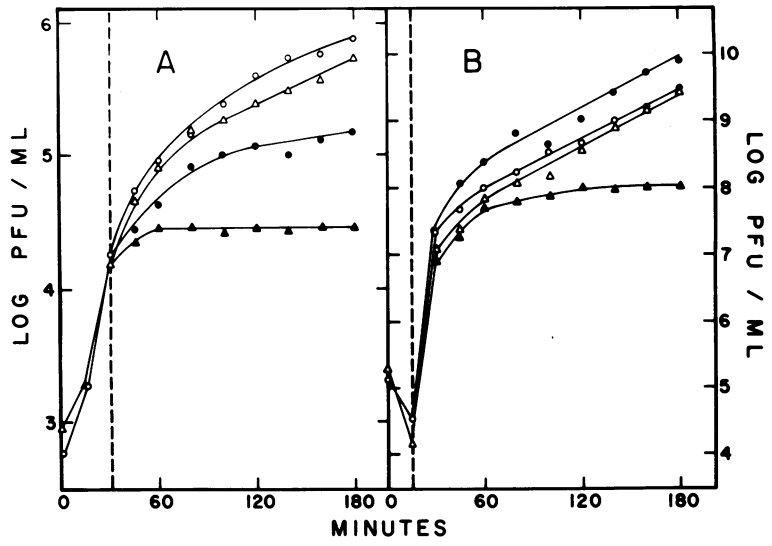

FIG. 2. *Growth of M13 and φX174am3 in dnaZts and TS⁺ cultures. (A) M13. Cultures of F'lac⁺/H727 dnaZts and TS⁺ were grown at 30°C in YET broth supplemented with 2 μg of thymine per ml to a density of 2 × 10⁸ cells/ml and concentrated to 2 × 10⁹ cells/ml in buffer (0.01 M potassium phosphate [pH 7.0] and 0.01 M MgSO₄). M13 was added (multiplicity of infection = 50), and the suspension was incubated for 30 min at 30°C. The cells were then washed twice in buffer and diluted to a concentration of 2 × 10³ cells/ml in YET broth (plus 2 μg of thymine per ml) at 30°C. After 30 min at this temperature, half of each culture was shifted to 41°C. Samples were taken at the times indicated and immediately plated on F'101/C600. dnaZts culture at 30°C (△) and at 41°C (▲); TS⁺ culture at 30°C (○) and at 41°C (●). (B) φX174. Cultures of C727 dnaZts and TS⁺ were grown at 30°C in YET broth supplemented with 1.5 mg of $CaCl_2$ per ml to a cell density of 2 × 10⁸ cells/ml, concentrated 10-fold into 0.01 M $MgSO_4$ and 0.01 M $CaCl_2$, and infected with φX174am3 (multiplicity of infection = 5) for 15 min at 30°C. The cells were then washed twice in the same solution and diluted to 2 × 10⁷ cells/ml in YET broth (containing 1.5 mg of $CaCl_2$ per ml) at 35°C. After 15 min, half of each culture was shifted to 40°C. Samples of 0.1 ml were transferred to 1.9 ml of lysis buffer (0.1 M Tris [pH 8.1], 0.5 mg of lysozyme per ml, and 10 mM EDTA) at the indicated times. Samples in lysis buffer were incubated for 60 min on ice, vortexed with a drop of chloroform, and titered on G727 TS⁺ (a host that can suppress the φX174am3 mutation). Symbols are as in (A).*

We conclude that the *dnaZ* function is required by both M13 and φX174 for continual phage production after the formation of parental RF DNA. It is unclear whether the differences in the rate of termination of M13 and φX174am3 phage growth at nonpermissive temperatures represent different requirements for the *dnaZ* product. Given the rapid rate of φX174 growth relative to that of M13, however, prolonged φX174 phage production at 40°C might reflect packaging of previously synthesized φX174 viral DNA or possibly slower inactivation of the *dnaZ* product at 40°C than at 41°C.

Requirement for the *dnaZ* function in M13 and φX174 RF replication. To determine which stages of phage DNA replication, after parental RF formation, were inhibited in the *dnaZ*ts mutants, we first examined RF → RF replication. F'*lac*⁺ derivatives of *dnaZ*ts and TS⁺ cultures were infected at 35°C with M13 and held at this temperature for 10 min. Pulse-labeling during this period demonstrated (Fig. 3A) the formation of RF DNA in both the mutant and revertant cultures at this permissive temperature. Duplicate cultures that were similarly infected and held at 35°C for 10 min were then incubated for an additional 15 min at either 35 or 41°C. Pulse-labeling the cultures that were held at 35°C revealed (Fig. 3B) RF and SS DNA synthesis in both ts and TS⁺ cultures; however, incubation at 41°C terminated M13-specific DNA replication in the *dnaZ*ts mutant (Fig. 3C).

The asymmetric synthesis of M13 SS DNA begins at approximately 10 min after infection (13). Therefore, it is possible that a significant amount of RF DNA present in Fig. 3B and C represented intermediates in SS synthesis. The inhibition of DNA synthesis in the ts culture at 41°C may thus reflect a *dnaZ* product requirement in asymmetric synthesis and not RF → RF replication. This ambiguity was resolved by treating M13-infected *dnaZ*ts and TS⁺ cultures

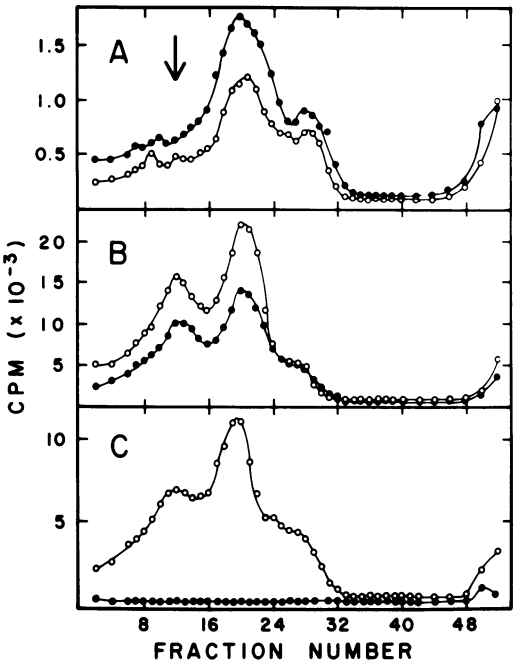

FIG. 3. *Replication of M13 RF DNA in dnaZts and TS⁺ cultures. F'lac⁺/H727 dnaZts and TS⁺ cultures were grown to 3.5 × 10⁸ cells/ml in YET broth supplemented with 2 μg of thymidine, transferred to 35°C, and infected with M13 (multiplicity of infection = 100). The vertical arrow indicates the position of ³²P-labeled M13 SS DNA marker. (A) dnaZts (●) and TS⁺ (○) cultures pulse-labeled for 10 min after infection at 35°C. (B) dnaZts (●) and TS⁺ (○) cultures, infected as above, were incubated at 35°C for 25 min after the addition of M13 and pulse-labeled for an additional 5 min with [³H]thymidine. (C) dnaZts (●) and TS⁺ (○) cultures were transferred to 41°C after a 10-min infection period at 35°C. Fifteen minutes after the temperature shift, the cultures were pulse-labeled for an additional 5 min.*

with chloramphenicol before pulse-labeling. Chloramphenicol, at concentrations of 10 to 30 μg/ml, selectively inhibits M13 SS DNA synthesis while permiting the replication of M13 RF DNA (14). Cultures of F'*lac*⁺ derivatives of *dnaZ*ts and TS⁺ strains were infected with M13 at 35°C and, after 10 min, a portion of each culture was shifted to 40°C. After an additional 10 min (20 min postinfection), chloramphenicol (30 μg/ml) was added to each of the cultures and the incubation was continued for 10 min. Pulse-labeling after chloramphenicol treatment demonstrated the synthesis of RF DNA in *dnaZ*ts and TS⁺ cultures at 35°C (Fig. 4A) and in the TS⁺ culture at 40°C (Fig. 4B) but virtually no M13 RF synthesis in the *dnaZ*ts culture at 40°C (Fig. 4B).

Similar results were obtained with φX174am3-infected cells. Chloramphenicol, at a concentration of 30 μg/ml, permits the formation and replication of φX174 RF DNA while inhibiting SS DNA synthesis (11). Chloramphenicol-treated (30 μg/ml) *dnaZ*ts and TS⁺ cultures of *E. coli* C were infected with φX174am3 at 35°C. A portion of each culture was shifted to 40°C after 5 min. During this 5-min infection interval at 35°C, RF I and II were formed (data not shown). After a 20-min incubation period at 35 or 40°C (25 min post-infection), the cultures were pulse-labeled for 5 min with [³H]thymidine. Incubation of the TS⁺ culture at 40°C resulted in a twofold increase of ³H incorporation into RF DNA over that obtained at 35°C, whereas incubation of the *dnaZ*ts culture at 40°C decreased incorporation to less than 3% of the level in a 35° C control culture (Table 1).

The *dnaZ* product is, therefore, required for RF replication by both M13 and φX174.

Requirement for the *dnaZ* function in M13 and φX174 SS DNA synthesis. To investigate the requirement for the *dnaZ* product in M13 SS synthesis, F'*lac*⁺ derivatives of *dnaZ*ts and TS⁺ cultures were infected at 30°C and held at this temperature for 120 min, followed by transfer of half of each culture to 41°C. After 15 min of additional incubation, the cultures were labeled with a 15-min [³H]thymidine pulse. Incubation at 41°C enhanced the incorporation of [³H]thymidine into both SS and RF DNA in the TS⁺ culture over that obtained at 30°C (Fig. 5A), but this treatment dramatically reduced the amount of incorporation into these DNA species in the *dnaZ*ts culture (Fig. 5B). The small amount of labeled SS and RF in the *dnaZ*ts culture that was incubated for 15 min at 41°C was completely eliminated by a 30-min incubation period at this temperature. DNA synthesis in the revertant culture was unchanged by this additional incubation at 41°C (data not shown).

A similar temperature effect was observed in φX174am3-infected *dnaZ*ts cultures (Table 2). ts and TS⁺ cultures were infected and incubated for 45 min at 30°C. Half of each culture was further incubated at either 30 or 40°C for 30 min, followed by pulse-labeling with [³H]thymidine for 5 min. As a result of the 30-min incubation at 40°C, the number of ³H counts that sedimented as SS φX174 DNA decreased, relative to the 30°C incubation data, by 16% in the revertant culture and by 94% in the *dnaZ*ts culture.

It is likely that the termination of M13 and φX174 SS synthesis is a direct result of a re-

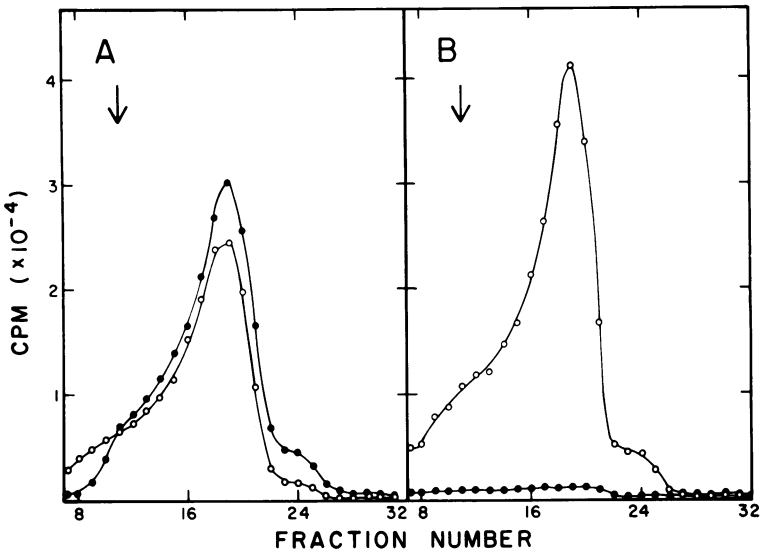

FIG. 4. *Replication of M13 RF in chloramphenicol-treated dnaZts and TS⁺ cultures. The vertical arrows indicate the position of M13 SS DNA. (A) dnaZts (●) and TS⁺ (○) cultures were infected as in Fig. 3 and incubated at 35°C for 20 min after the addition of M13. The cultures were then treated with chloramphenicol (30 μg/ml) for 10 min, followed by pulse-labeling for 5 min with [³H]thymidine. (B) dnaZts (●) and TS⁺ (○) cultures were transferred to 40°C 10 min after infection at 35°C. Ten minutes after the shift to 40°C, chloramphenicol (30 μg/ml) was added and the 40°C incubation was continued for an additional 10 min. The cultures were then pulse-labeled for 5 min with [³H]thymidine.*

TABLE 1. *Replication of φX174am3 RF in dnaZts and TS⁺ cultures*[a]

Host	Total RF I and II synthesis (cpm)		
	35°C	40°C	Ratio 40/35°C
*dnaZ*ts	12,100	300	0.025
TS⁺	10,700	22,800	2.1

[a] C727 *dnaZ*ts and TS⁺ cultures were grown to 3 × 10^8 cells/ml in YET broth (containing 1.5 mg of $CaCl_2$ per ml) at 30°C, concentrated 10-fold into 0.01 M $MgSO_4$ and 0.01 M $CaCl_2$, and infected with φX174am3 (multiplicity of infection = 3) at 35°C. After 10 min, the cells were diluted 1:10 into YET broth (plus 1.5 mg of $CaCl_2$ per ml) containing 30 μg of chloramphenicol per ml and incubated for 5 min at 35°C. Half of each culture was transferred to 40°C, and the incubation was continued for 20 min. All of the cultures were then pulse-labeled with [³H]thymidine for 5 min at their respective temperatures, harvested, lysed, and analyzed. The data presented for each culture are the sum of the trichloroacetic acid-insoluble ³H counts that sedimented as RF I and II DNA.

quirement for the *dnaZ* product in this reaction rather than a secondary effect of the *dnaZ* requirement in RF replication with a subsequent depletion of a necessary RF pool. M13 RF replication, but not SS synthesis, requires the *dnaG* product (15); a requirement for the *dnaC* product has been observed in φX174 RF replication but not in SS synthesis (11). In both of these examples, shifting phage-infected temperature-sensitive cultures of these mutants to nonpermissive temperatures after RF replication permitted large amounts of SS synthesis that were comparable either to those observed in revertant cultures at that temperature (15) or to those obtained in the mutant cultures at a permissive temperature (11). Shifting M13- or φX174-infected *dnaZ*ts cultures to nonpermissive temperatures after RF replication dramatically curtailed SS synthesis relative either to mutant cultures at a permissive temperature or to revertant cultures at the elevated temperature.

Thus, it appears that the synthesis of M13 and φX174 SS DNA requires an active *dnaZ* product.

DISCUSSION

Based on the data in this communication and those previously reported (10), it appears that the *dnaZ* function is required by both M13 and φX174 in all stages of their DNA replication cycle. Conversion of SS DNA to RF, RF replication, and the synthesis of progeny single strands are inhibited in *dnaZ*ts mutants at nonpermissive temperatures. The finding that an active *dnaZ* product is dispensable for a loss in rifampin sensitivity in the M13 SS → RF

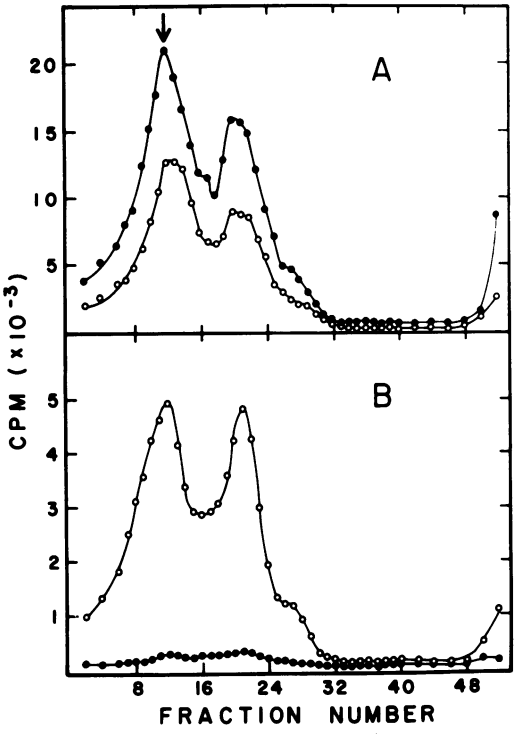

FIG. 5. *Synthesis of M13 single-strand DNA in dnaZts and TS⁺ cultures. F'lac⁺/H727 dnaZts and TS⁺ cultures were grown to 3 × 10⁸ cells/ml in YET broth (containing 2 μg of thymidine per ml) at 30°C and infected with M13 (multiplicity of infection = 100). After a 30-min infection period, the cells were diluted 1:4 into fresh YET broth (containing 2 μg of thymidine per ml) and the incubation was continued for 90 min. The vertical arrow indicates the position of M13 SS DNA. (A) TS⁺ culture. After the 90-min incubation period, half of the culture was transferred to 41°C. Both subcultures were incubated for 15 min and then pulse-labeled with [³H]thymidine for an additional 15 min. Symbols: (○) 30°C; (●) 41°C. (B) dnaZts culture treated as in (A). Symbols: (○) 30°C; (●) 41°C.*

conversion indicates that the *dnaZ* function is not needed for RNA priming and predicts that it would be required for DNA synthesis on RNA-primed templates. Wickner and Hurwitz have recently demonstrated, by using an in vitro complementation assay, that the *dnaZ* product is an elongation factor required by DNA polymerase III for duplex DNA synthesis on RNA-primed SS phage templates (21). In the in vitro reaction, using purified proteins, the *dnaZ* product was needed for nucleotide incorporation on primed templates by DNA polymerase II and III but was not absolutely required by DNA polymerase I (21).

The role of the *dnaZ* product in DNA polymerase III activity does not appear to be confined to parental RF formation. Replication of RF DNA and the asymmetric synthesis of viral single strands, processes requiring an active *dnaE* product (3, 8, 18), terminate when the *dnaZ*ts product is inactivated. As with parental RF formation, however, our data do not distinguish between a requirement for the *dnaZ* function in ongoing polymerization and in an initiation event in DNA synthesis that may follow RNA priming. φX174 RF replication and SS synthesis terminate in the absence of the *E. coli dnaG* product (12), which has been reported to possess rifampin-insensitive RNA polymerase activity (1). M13 RF replication requires the *dnaG* product (15) and is also inhibited by rifampin (6). M13 SS synthesis does not require the *dnaG* product (15) and is rifampin resistant for at least two successive rounds of SS synthesis (6). It could not be determined, however, whether a rifampin-sensitive initiation event was required after several rounds of SS synthesis (6). Thus, it is possible that all stages of M13 and φX174 DNA replication require RNA priming events. It therefore follows that the *dnaZ* product may play a role in the initiation of the DNA polymerization reaction (e.g., facilitating the formation of a DNA polymerase/primer complex) rather than being needed for the actual polymerization reactions involved. The observation that DNA polymerase III, without elongation factors, is able to incorporate nucleotides on DNase-treated (21) or gapped duplex templates (22) makes this possibility more attractive.

TABLE 2. *Synthesis of φX174am3 SS DNA in dnaZts and TS⁺ cultures*[a]

Host	SS DNA synthesis (cpm)		
	30°C	40°C	Ratio 40/30°C
*dnaZ*ts	55,600	3,500	0.06
TS⁺	66,600	56,200	0.84

[a] C727 *dnaZ*ts and TS⁺ cultures were grown and concentrated as in Table 1 and infected with φX174am3 (multiplicity of infection = 3) at 30°C for 15 min. The cells were diluted 1/10 into YET broth (containing 1.5 mg of $CaCl_2$ per ml) and incubated for 45 min at 30°C. Half of each culture was then transferred to 40°C, incubation was continued for 30 min, and all of the subcultures were pulse-labeled with [³H]thymidine for 5 min. The ³H incorporation into SS DNA was determined as in Table 1.

ACKNOWLEDGMENTS

We thank the individuals indicated for strains.

This work was supported by American Cancer Society grant NP169 and, in part, by Public Health Service grant

AI08286 from the National Institute of Allergy and Infectious Diseases. W.G.H. was supported by Public Health Service training grants 5 T01 GM00337 and 1 T32 GM07126, both from the National Institute of General Medical Sciences; J.R.W. was supported during a portion of this work by Public Health Service research career development award GM29413 from the same Institute.

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