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Replication of Bacteriophage φX-174 in a Mutant of *Escherichia coli* Defective in the *dna*E gene

(DNA polymerases/temperature-sensitive mutants/φX-174 DNA)

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ABSTRACT **Strains of *E. coli* bearing a temperature-sensitive mutation at the *dna*E locus were unable to support growth of bacteriophage φX-174 at 43° while permitting normal growth at 30°. The *dna*E gene product was implicated in all stages of φX-174 DNA replication. Differences were observed between a double mutant (*pol* A$^-$, *dna*E^{ts}) and a single mutant (*pol* A$^+$, *dna*E^{ts}) in the ability to synthesize parental replicating form and its subsequent replication. The implications of these observations are discussed in terms of the relationship between DNA polymerases-I and -III.**

Replication of bacteriophage φX-174 DNA is accomplished in three temporally and mechanistically distinct stages. The first stage, formation of a double-stranded "replicating form (RF)" by synthesis of a complementary strand of the infecting single strand of the virus, is accomplished by host-cell enzymes (1). The second stage, semi-conservative RF replication, requires at least one phage function and an undetermined number of host functions. The third stage, conservative displacement synthesis of progeny viral strands from a RF precursor, is also dependent on both phage and host functions (2).

There are three presently known DNA polymerases of *Escherichia coli* (3). Mutants defective in either polymerase-I (4) or -II (5, 6), or both, are able to support the growth of φX-174. Temperature-sensitive mutants at the *dna*E locus have been shown to contain a thermolabile polymerase-III (7). This paper presents results of a study of φX-174 growth in an *E. coli* strain temperature-sensitive at the *dna*E locus in order to determine which stages of φX-174 DNA replication are blocked under restrictive conditions and therefore require a functional gene *E* protein.

MATERIALS AND METHODS

Bacterial and Phage Strains. *E. coli* BT1000 pol A1$^-$, *endo* I$^-$, thy$^-$, φX^s, and BT1026, a *dna*E^{ts} derivative of BT1000 (8), were kindly provided by Dr. F. Bonhoeffer. Strain T14 is a pol A1$^+$ transductant of BT1026 that was selected for methyl-methane sulfonate (MeMS) resistance and temperature-sensitivity in colony formation and [^{3}H]-thymine incorporation. Crude extracts of T14 had over 100 times as much DNA polymerase activity as BT1026, by the assay method of Kornberg and Gefter (3). Strain HF4726 is a permissive strain for plating φXam3.

φXam3 is a nonlysing mutant of φX-174 (9). Although this phage has been reported not to grow above 42°, I have found the upper temperature limit of growth to be host-strain dependent. In BT1000, φXam3 grew well at up to 44°. Transducing phage Plkc was grown on *E. coli* JC1557 (pol A1$^+$) and kindly provided by Dr. C. Lark.

Media. TKBT medium contained 10 g/liter of tryptone, 5 g/liter of KCl, 0.074 g/liter of $CaCl_2 \cdot 2H_2O$, and 2 mg/liter of thymine. L-Tris agar plates contained 10 g/liter of tryptone, 5 g/liter of yeast extract, 5 g/liter of NaCl, and 20 g/liter of agar, dissolved in 0.01 M Tris·HCl (pH 7.5). After autoclaving, 10 ml of 0.1 M $MgCl_2$ and 10 ml of 0.25 M $CaCl_2$ were added per liter. 0.06 ml of MeMS per 100 ml of L-Tris agar was added to plates after pouring and the plates were used the same day.

Transductions. The method of Lennox (10) was used for transduction. Recipient cells were grown to a density of 2×10^8 cells per ml and infected with a multiplicity of 3.0.

Infections and Preparation of DNA. Cells were grown at 30° to a density of 1–2 $\times$ 10^8/ml, transferred to 43°, incubated for 20 min, and infected with ^{32}P-labeled, 5-bromouracil-substituted φXam3 at a multiplicity of 1.0. Samples were rapidly chilled by dilution in −70° acetone, and the cells were collected by centrifugation. In the replication experiment (Fig. 2), washing, lysis, and removal of *E. coli* DNA were performed as described by Franke and Ray (11), with a high-salt sucrose gradient. Fractions containing [^{32}P]RF were pooled, dialyzed, and centrifuged to equilibrium in CsCl together with markers of φXtsγ_h DNA and ^{3}H-labeled *E. coli* DNA (the latter kindly provided by K. G. Lark).

Assays. Measurement of acid-precipitable counts was made by the method of Barnett and Jacobson (12).

Infectivity of φXam3 and φXtsγ_h DNA was performed by the Guthrie and Sinsheimer procedure (13), and differential plating for the two mutants was described (14) with HF4726 as the permissive strain.

RESULTS

At 43°, *E. coli* BT1000 supported the growth of φXam3 but strains BT1026 and T14 did not. All strains supported growth of φXam3 at 30°. When BT1026 or T14 were infected at 30° and subsequently shifted to 43°, the course of progeny phage production was blocked regardless of when the shift-up was made.

In order to determine if parental RF could be formed at 43°, a culture of BT1026 was grown at 30°, shifted to 43°, and,

Abbreviations: RF, replicating form; MeMS, methyl-methane sulfonate.

after a 20-min incubation at 43°, infected with [^{32}P]ϕXam3. DNA from infected cells was extracted with phenol and centrifuged to equilibrium in CsCl (see Fig. 1). Conversion of single-stranded DNA to RF was observed, as indicated by the appearance of [^{32}P]DNA at the same density position as [^{14}C]RF marker. In this experiment a rather high proportion of unconverted single strands remained, which was also found in a control infection at 30° and was probably due to a high proportion of inactive phage particles in the preparation. However, the RF formed at 43° in BT1026 was slightly shifted toward higher density, suggesting a possible structural defect.

The ability of cells to replicate RF at 43° was tested by a density-shift experiment with density-labeled parental phage. Cultures incubated 20 min at 43° were infected with [^{32}P]-BrdU-ϕXam3 for an additional 20-min period. After lysis and purification on a high-salt sucrose gradient, the RF was centrifuged to equilibrium in CsCl. Replication of RF would be indicated by the appearance of ϕXam3 infectivity at the density position of fully light RF. Fig. 2 shows the results of such an experiment, and the data comparing fully light (progeny) infectivity with parental ^{32}P counts are given in Table 1. Strain BT1026 formed less than 1/200th of the progeny RF formed normally (BT1000). However, it can be seen that strain T14 formed 18-times as much RF as strain BT1026. Therefore, the introduction of a temperature-sensitive *dna*E lesion into a pol-I$^-$ strain (BT1000) normally able to replicate RF resulted in inability to replicate RF (BT1026). However, the reintroduction of an active pol-I function (T14) resulted in partial compensation and the ability to carry out an appreciable, albeit sub-normal, amount of RF replication.

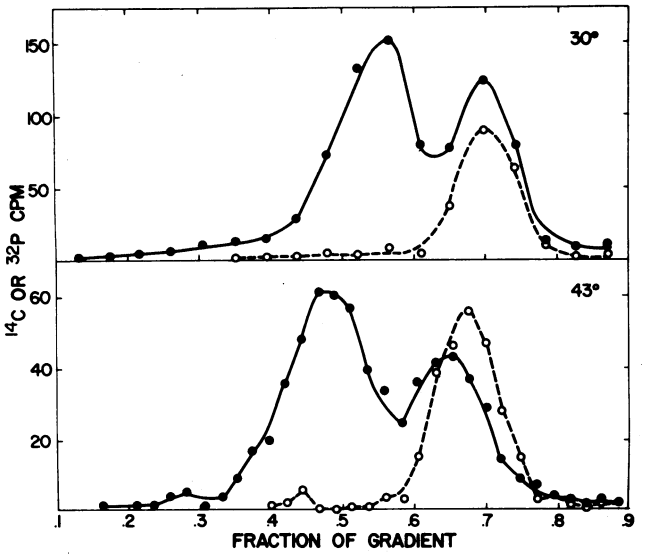

FIG. 1. Formation of parental RF in BT1026. A culture of BT1026 was grown to a density of 1–2 × 10^8 in TKBT (see *Methods*) at 30°, and half of the culture was then transferred to 43°. After an additional 20-min incubation, both cultures were infected with [^{32}P]ϕX-174 at a multiplicity of 1.0. 10 min after infection the cultures were chilled, pelleted, and lysed, and DNA was extracted with phenol. Equilibrium CsCl gradients were run at 20°, with purified [^{14}C]RFI as a density marker. Fractions were collected on Whatman 3 MM paper discs, and acid-precipitable counts were measured by the method of Barnett and Jacobson (12). ^{32}P, ●——●; ^{14}C, O- - -O.

It was observed that the specific infectivity of parental RF in BT1026 was at least 4-fold lower than that of T14 or

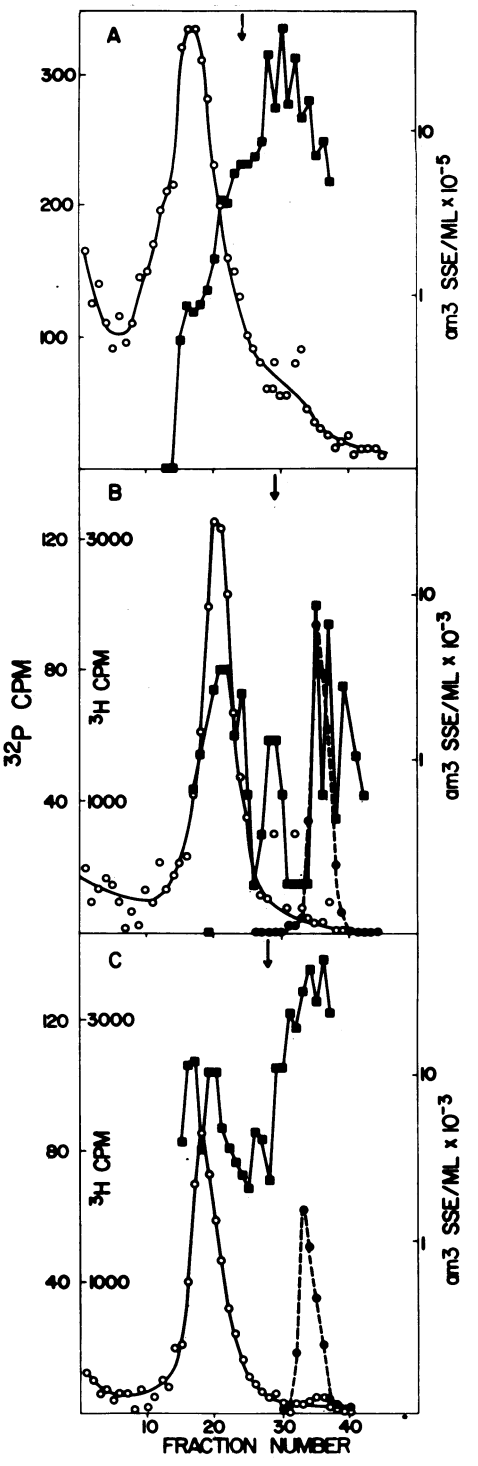

FIG. 2. RF replication experiment. Cultures of BT1000 (*A*), BT1026 (*B*), or T14 (*C*) were grown and infected, and RF was purified as described in *Methods*. (Culture of BT1000 was harvested 10 min after infection.) The RF was centrifuged for 40 hr in CsCl ($\rho_{average}$ = 1.7) at 20°, 37,000r pm (150,000 × *g*), together with markers of ϕX$^{ts}\gamma_h$ single-stranded DNA and *E. coli* [^{3}H]DNA. After drop collection, 0.01 ml of each fraction was removed for infectivity assay and 0.1 ml was used for determining acid-precipitable counts. O——O, ^{32}P counts; ●- - -●, ^{3}H counts; ■——■, am3 infectivity. The peak of the band of γ_h infectivity is given by the *arrow*. SSE, single-strand equivalents.

BT1000. This difference may be related to the structural alteration observed in Fig. 1.

The ability of cells infected at 43° to resume phage production at 30° was measured in a temperature shift-down experiment. Cells grown at 30°, shifted to 43° for 20 min, and infected with φXam3 were shifted back to 30° at 10, 15, 20, and 30 min after infection. The time-course of intracellular phage production under these conditions is shown in Fig. 3. In strain T14, cells shifted to 30° at 10, 15, or 20 min after infection all commenced phage production at about 30 min, whereas cells shifted down at 30 min were delayed an additional 10–15 min. This result would be expected if the course of phage infection at 43° was not blocked until the end of the latent period. In contrast, the onset of phage production in strain BT1026 was delayed by an amount corresponding to the length of time the infected cultures were held at 43°. These cells, therefore, were blocked early in the latent period at 43°. An additional recovery period at 30° was necessary for both strains, presumably due to a need to synthesize active *dna*E gene product. Although strain BT1026 was reported to resume DNA synthesis after a 7-min heat pulse at 45° (8), this presumption is supported by the observation in this laboratory that cells shifted to 43° for 40 min were unable to resume normal [^{3}H]thymine incorporation at 30° in the presence of chloramphenicol. Control infected cultures, left at 43°, failed to make detectable intracellular phage.

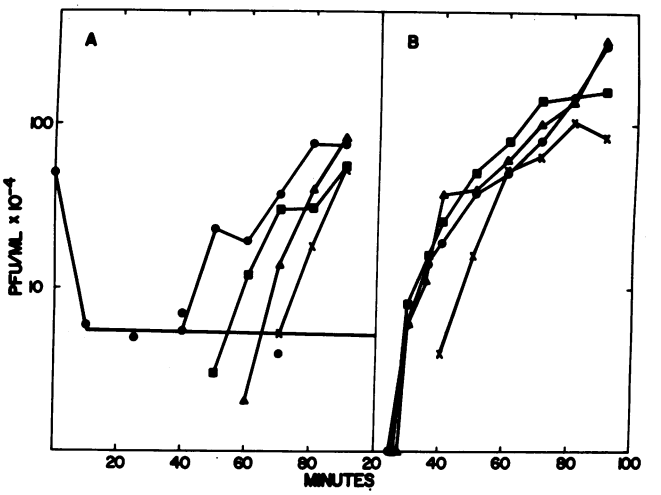

FIG. 3. Temperature shift-down experiment. Cultures of BT1026 (*A*) or T14 (*B*) were grown at 30° in TKBT to a density of 1–2 × 10^8/ml, transferred to 43°, and incubated for another 20 min. The cells were infected with φXam3 at a multiplicity of 0.01 and diluted 10-fold into warmed TKBT 10 min after infection. Portions of the infected culture were shifted to 30° at 10 (●——●), 15 (■——■), 20 (▲——▲), and 30 (×——×) min after infection, and a portion was kept at 43° (○——○) as a control. PFU, plaque-forming units.

DISCUSSION

Two strains of *E. coli* bearing a temperature-sensitive lesion at the *dna*E locus were unable to support the growth of bacteriophage φX-174 at 43°. Both strains were blocked in progeny phage production. At any time after infection, a shift from permissive to restrictive temperature resulted in cessation of phage production. The *dna*E function is therefore specifically required for φX-174 replication.

The role of the *dna*E function in each stage of φX-174 DNA replication has been investigated. These will be discussed in reverse order of occurrence, beginning with the third stage, single-stranded DNA synthesis and progeny phage production. Both *dna*E mutants tested were blocked after a shift-up to restrictive temperature during progeny phage production. The *dna*E function is therefore specifically required for this stage of infection, presumably due to its role in single-strand replication.

TABLE 1. *Progeny RF infectivity compared to parental ^{32}P counts**

Strain	Parental RF		Progeny RF		Progeny/parental ratio (single-strand equivalents, ^{32}P cpm)
	Fractions	^{32}P cpm	Fractions	Single-strand equivalents	
BT1000 (*pol A*$^-$)	14–20	2025	28–34	1.75 × 10^7	8642
T14 (*dna*E^{ts})	15–21	396	31–37	2.55 × 10^5	644
BT1026 (*pol A*$^-$, *dna*E^{ts})	17–24	667	34–42	2.43 × 10^4	36

* Data from Fig. 2.

The role of the *dna*E function in RF replication appears to be more complex. Both strains tested made subnormal amounts of progeny RF (Table 1), compared to the control strain BT1000 (*pol*A$^-$, *dna*E$^+$). It therefore appears that the *dna*E function is used for RF replication also. A significant difference was noted, however, between the two *dna*E^{ts} strains. The single mutant, T14 (*pol* A$^+$, *dna*E^{ts}), was inhibited, but was able to make 18-times as much progeny RF as the double mutant, BT1026 (*pol* A$^-$, *dna*E^{ts}). This amounted to about 4 RF per cell, based on a comparison of parental to progeny infectivity, while the double mutant was virtually unable to replicate RF at 43°. This difference appears to be biologically significant, as shown by Fig. 3, which demonstrates a dramatic difference between the single and the double mutant in their behavior after a temperature shift-down. From this experiment it can be concluded that for φX-174 replication the single mutant is not completely inhibited at 43° until the end of the eclipse period, whereas in the double mutant inhibition occurs early in infection. Thus, for RF replication, the presence of active DNA polymerase-I appears to partially compensate for the loss of the *dna*E function, DNA polymerase-III (7). The mechanism of this compensation is not understood. Possibly the presence of residual polymerase-III activity is necessary before polymerase-I can act.

In the conversion of the parental single strand to RF, the *dna*E function is also implicated. This is shown by comparison of BT1000 (*pol* A$^-$, *dna*E$^+$), which made a parental RF with normal density and specific infectivity, with the double mutant, BT1026 (*pol* A$^-$, *dna*E^{ts}), which formed parental RF having anomalous density and reduced specific infectivity. A requirement for polymerase-III for φX-174 DNA-dependent [α-^{32}P]dTTP incorporation *in vitro* has been reported by Wickner *et al.* (15). The data reported here appear to be somewhat at variance with their findings, inasmuch as the formation of RF *in vivo* does not appear to be inhibited by

high temperatures to the same extent. Since the activity of BT1026 has been observed to be more temperature sensitive *in vitro* than *in vivo* (8), the possibility exists that residual polymerase-III activity could account for the observed RF formation *in vivo*. In the single mutant, T14 (*pol* A^+, *dnaE*^{ts}), the parental RF formed had normal specific infectivity, a 4-fold increase over that in the double mutant. It would then appear that polymerase-I may partially substitute for polymerase-III in this stage of replication as well.

These experiments indicate that the *dnaE* function (DNA polymerase-III) is involved in both early and late stages of ϕX-174 replication. The effects of temperature-inactivated polymerase-III on RF formation and replication can be partially compensated by the presence of active DNA polymerase-I. Such compensation is not observed, however, in the single-strand synthesis stage of ϕX-174 replication, where DNA polymerase-III is specifically required.

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