Rescue of DNA Replication and Bacteriophage Production After Infection with T4 DNA Ligase Mutants

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By preventing phage DNA synthesis during a critical period, conditions have been found under which DNA replication and phage production are rescued after infection with T4 DNA ligase mutants.

The Okazaki model of replication postulates a discontinuous way of DNA synthesis with subsequent ligation of primary polynucleotide segments into the daughter strands of replicated DNA. Part of the supporting evidence for this model has come from experiments with T4 ligase (gene 30) mutants which show small T4 DNA pieces accumulating under nonpermissive conditions (7). Under permissive conditions these pieces eventually enter whole T4 DNA molecules.

The above model ascribes a fundamental role(s) to ligase(s) in DNA replication. In fact, T4 ligase amber mutants (gene 30) show reduced synthesis of viral DNA (only a few phage equivalents of DNA) and yield very few viable phages (≤ 0.01 phage per cell).

We have found conditions under which cells infected with DNA ligase amber mutants are able to replicate T4 DNA effectively and to produce viable phages.

Escherichia coli B^E cells were infected at 30 C with the double mutant *tsP36-amH39X* (gene 43: DNA polymerase, gene 30: DNA ligase). It has been previously shown that the *tsP36* product (DNA polymerase) is reversibly inactivated by temperature shift (8). Cells were kept at 30 C for the first 10 min after infection, and then the temperature was shifted to 42 C for 10 more min ($t = 20$ min) and then lowered back to 30 C. Under these conditions, DNA synthesis, measured as ¹⁴C-thymidine incorporation into alkali-resistant acid-precipitable material, has the pattern shown in Fig. 1. The corresponding phage yields (burst

size from single cells), measured 90 min after infection, are shown in Table 1. A significant rescue of both T4 DNA synthesis and viable phage production is observed upon shift-down to 30 C at 20 min after infection. The same results have been observed in another mutant in gene 30 (*amE605*).

Although under the abovementioned conditions phage yield and DNA synthesis are rescued, most of this DNA is in an unstable form. In fact, if in the experiments described in Fig. 1 a portion of the culture is again shifted up to 42 C at 30 or 40 min after infection, extensive DNA degradation is observed (Fig. 1, curves D and E). The extent of this degradation is so great that it probably involves all the DNA synthesized during the second period at low temperature (Fig. 1, curve C).

The instability of the rescued DNA confirms a previous finding on *tsP36-tsA80* (genes 43, 30) infected cells (9). Also in this case, extensive degradation of the DNA synthesized under permissive conditions is observed upon temperature inactivation of ligase and DNA polymerase, proving that in the absence of DNA synthesis, phage ligase is continuously required for the stability of phage DNA.

When the same experiment is repeated with different times of shift-up (15 min after infection) and shift-down (25 min after infection), a drastic decrease both in T4 DNA synthesis rescue and phage yield is observed (data not shown).

In the experiment described in Fig. 1, chloramphenicol (CAP) addition (200 μ g/ml) at 19 min after infection (just before shift-down) had no effect on the rescue of DNA synthesis, but if CAP was added 9 min after infection (just before shift-up), DNA syn-

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FIG. 1. Fate of the T4 DNA synthesized during infection with *tsP36-amH39X* infection as a consequence of reversible inactivation of the *tsP36* DNA polymerase. *E. coli B^E* grown in M9S to 5×10^8 /ml were infected at 30 C with 8 phages per bacterium. ^{14} C-thymidine (0.05 μ Ci and 20 μ g/ml) was added 3 min later. A portion of the culture was shifted to 42 C ($\uparrow$) and later to 30 C ($\downarrow$) at the times shown. At various times ^{14} C incorporation into Cl_3CCOOH insoluble linkages was measured after overnight digestion at 30 C in 0.5 M NaOH. Symbols: $\circ$, incorporation at 30 C; $\bullet$, incorporation at 42 C. Infection at 30 C (curve A); part of the culture is shifted up to 42 C (curve B) and subsequently shifted down to 30 C (curve C). Portions of the cells after shift-down are shifted up again to 42 C at different times (curves D and E). Bacterial growth, infection conditions, phage stock preparations, multiple mutant constructions, labeling addition, and plating conditions have been published in detail elsewhere (2).

TABLE 1. Phage yields in the *tsP36-amH39X* infection due to reversible inactivation of the *tsP36* DNA polymerase^a

Mutant	30 C	$\uparrow$ 42 C	$\uparrow$ 42 C $\downarrow$ 30 C
<i>tsP36-amH39X</i>	0.1	0.0001	12
<i>amH39X</i>	0.05	0.1	0.0001

^a *E. coli B^E* (su^-) grown in M9S to 5×10^8 /ml were infected with the appropriate phage mutant at 30 C with 8 phages per bacterium. Phage yields were measured 90 min after infection by addition of CHCl_3 and plating on CR63 (su_1^+) at 30 C. The first column (compare curve A, Fig. 1) shows the phage yield in the infection at 30 C, the second column (curve B, Fig. 1) shows the phage yield when part of the culture is shifted to 42 C 10 min after infection, and the third column (curve C, Fig. 1) shows the phage yield after subsequent shift-down to 30 C at 20 min after infection. The numbers represent viable phages per bacterium.

thesis was not rescued after the subsequent shift-down.

From the experiments described above it appears that if DNA synthesis is arrested

during a critical time period, the absolute requirement for T4 ligase in further DNA synthesis is lost.

This conclusion receives further support from the results of the following experiment: cells were infected with the mutant *tsP36-amH39X* at 42 C, and 8 or 15 min after infection the temperature was shifted down to 30 C. Linear DNA synthesis occurred only when the shift-down was made 15 min after infection (Fig. 2, curve C); when the shift-down was made at 8 min, the normal DNA-arrested phenotype of ligase mutants was maintained (Fig. 2, curve B).

At the same time, the experiments with CAP suggest that some phage-coded protein(s), required for DNA replication in the absence of ligase, must be synthesized during this critical period.

Our results, therefore, argue against any absolute requirement for the function of gene 30 product (phage-coded DNA ligase) in T4 replication. Others have presented similar conclusions. Kozinski (5) has shown that addition of CAP at early times (3–5 min after infection at 37 C) partly rescues DNA synthesis in an amber mutant in gene 30 and that, upon CAP removal, maturation takes place and 10 to 30 phages per cell are produced. He interprets these results as evidence that ligase is an antagonist of an (unspecified) en-

FIG. 2. T4 DNA synthesis during the infection with *tsP36-amH39X* after reactivation of the DNA polymerase. Cells were infected at 42 C, and at the times indicated ($\downarrow$) portions of the culture were cooled down to 30 C. Infection at 42 C (curve A); cells shifted to 30 C 8 min after infection (curve B); cells shifted to 30 C 15 min after infection (curve C). Cell growth, infection, labeling conditions, and ^{14} C incorporation are the same as described in the legend to Fig. 1. The residual ^{14} C incorporation shown in the 42 C infection (curve A) is host DNA synthesis which occurs after T4 ligase mutant infection (2).

donuclease that acts on DNA at a critical time. More recently, several authors (1, 3, 4, 6) have shown that the requirement for T4 ligase in viral DNA replication is suppressed by a second mutation, in gene rII. In double mutant (gene 30⁻, gene rII⁻) infected cells, they observed delayed but otherwise abundant DNA synthesis and normal phage yield; to rescue DNA synthesis and viable phage production, it is sufficient that the rII gene product be inactivated in the early stage of infection only (Karam, *personal communication*).

A tentative explanation for these findings can be proposed: gene 30 product is required in a stoichiometric amount to antagonize an early phage-induced endonuclease which is active on both parental and newly replicated DNA. If this is the case, the amount of irreversible damage to T4 DNA produced by this endonuclease (i.e., double-strand cuts derived from the replication of single-strand nicked DNA in the absence of ligase) could be limited (i) by preventing abundant DNA replication upon CAP addition at very early times, which decreases the rate of T4 DNA synthesis several-fold; (ii) by introducing an rII mutation which probably decreases nuclease activity (10); or (iii) by inhibiting replication with a temperature-sensitive mutation in the structural gene of the phage-coded DNA polymerase. The T4 gene 30 product would be required only at the beginning of the infection to antagonize the endonuclease action. If we can prevent this endonuclease from irreversibly damaging T4 DNA, then the gene 30 product is no longer absolutely required and other ligase(s) (i.e., host ligase) may eventually be used to ensure effective phage DNA replication and phage yield.

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