

Replication of Bacteriophage ϕ X174 in a Temperature-Sensitive Deoxyribonucleic Acid Host Cell

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Bacteriophage ϕ X174 is unable to replicate in *Escherichia coli* t3 at the restrictive temperature. However, if progeny phage synthesis is initiated at the permissive temperature, it will continue after a shift to the restrictive temperature.

Studies on the replication of small bacteriophages, such as ϕ X174, in temperature-sensitive (*ts*) deoxyribonucleic acid (DNA) strains are important for two reasons. First, since the DNA of these phages contains only 5,500 nucleotides (8, 12), they probably possess no more than 10 genes, of which nine have been identified (4, 5). With such limited genetic content, these phages might utilize host cell enzymes for replication of their DNA. Evidence for this has been provided by the observation that the early stages in ϕ X replication occur in the presence of 150 μ g of chloramphenicol per ml (14) and by the isolation of host cell mutants which permit the adsorption of these phages but not their subsequent replication (2). A further definition of the host-mediated steps in viral replication should be possible by determining whether small phages are capable of replicating in *ts*DNA strains.

Second, numerous investigators have isolated *ts*DNA strains of *Escherichia coli* (1, 3, 6, 7, 9) but, with one possible exception (9), the thermolabile proteins have not been identified. Since the replication of these small phages involves several discreet steps which have been characterized (12), it may be possible to identify the defective enzyme by observing what steps in replication are blocked at the restrictive temperature. Recently, we attempted such an analysis of the two *ts*DNA strains isolated by Bonhoeffer (10; Primrose & Dowell, unpublished data; Primrose, Ph.D. Thesis, Univ. of California at Davis, 1969).

Sinsheimer et al. (13) have reported similar studies on another *ts*DNA strain, *E. coli* t3 infected with ϕ X174. These workers found that the formation and replication of the first replicative form were blocked in this strain at the restrictive temperature (42 C). Furthermore an immediate cessation of phage synthesis was ob-

served if infected cells which were synthesizing new phage particles at the permissive temperature were shifted to the restrictive temperature. However, these workers used ϕ X174*am*3 which has reduced replication at temperatures above 40 C (C. A. Hutchinson III, Ph.D. Thesis, Calif. Institute of Technology, 1969; C. E. Dowell and D. L. Dapper, *in preparation*), and this raises the possibility that the observed shut-off of phage production at 42 C was due not to the thermosensitive lesion in the host cell but to the inherent inability of the phage to replicate well at this temperature (Fig. 1). Consequently, we have re-examined the replication of ϕ X174 in this strain by using a phage mutant, ϕ X_{ahb} (Primrose, Ph.D. Thesis; Dowell and Dapper, *in preparation*; Primrose and Dowell, *in preparation*), which can replicate at higher temperatures than ϕ X174*am*3 (Fig. 1).

From the data presented in Fig. 2, it is clear that a block to ϕ X replication occurs in strain t3 since cells shifted to 42 C shortly after infection fail to produce phage; however, when phage production has begun at 35 C, a shift to 42 C has little effect. The continued synthesis of phage after the shift could result either from depletion of a pool of viral single strands synthesized prior to the shift or from de novo synthesis after the shift. The first of these possibilities is not too likely since an intracellular pool of free viral single strands has never been found in ϕ X-infected cells (13). Evidence for de novo synthesis of single strands can be obtained by shifting infected cells into a medium containing labeled thymine since de novo synthesis of single strands demands that label be incorporated into progeny phage particles at 42 C, whereas no label incorporation should be observed if an intracellular pool of single strands is being depleted. Infected

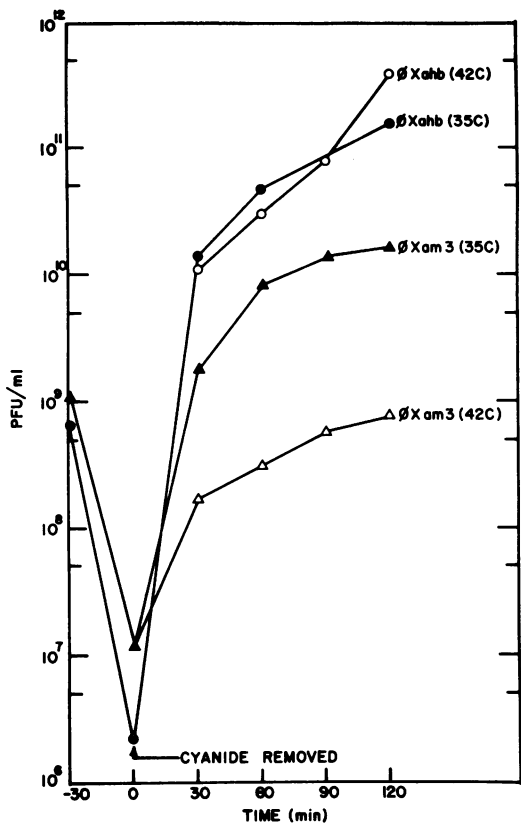


FIG. 1. Replication of $\phi X174am3$ and $\phi Xahb$ at 42 C in *E. coli* TAU bar. Cells of *E. coli* TAU bar were grown at 35 C in TPG2A to 3×10^8 per ml, centrifuged, and resuspended in starvation buffer containing 0.01 M Ca^{2+} and 0.009 M KCN. Phage were added at a multiplicity of infection of 3. After 30 min, the culture was diluted 1,000-fold into TPG2A warmed to 35 or 42 C. Samples were removed at regular intervals and assayed for intracellular phage.

cells show a marked incorporation of label at 42 C which parallels phage production, and sedimentation of the phage produced after the shift (Fig. 3) reveals coincident peaks of radioactivity and phage infectivity, confirming that labeled phage were produced.

E. coli t3, which is a *ts*DNA derivative of *E. coli* 15 TAU bar, was kindly supplied by K. Rasmussen, University Institute of Microbiology, Copenhagen. An identical culture was also supplied by R. L. Sinsheimer, California Institute of Technology, Pasadena. *E. coli* 15 TAU bar, originally from Copenhagen, was supplied by J. L. Ingraham, University of California at Davis. Both strains require thymine, arginine, uracil, tryptophan, methionine, and proline for growth and are su^- .

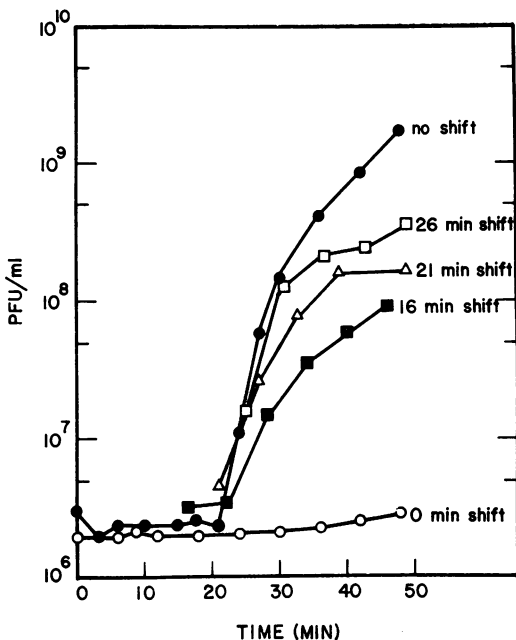


FIG. 2. Production of $\phi Xahb$ in strain t3 at 42 C. Cells of *E. coli* t3 were grown at 35 C in TPG2A to 3×10^8 per ml, centrifuged, and resuspended in starvation buffer containing 0.01 M Ca^{2+} and 0.009 M KCN (to synchronize infection). $\phi Xahb$ was added at a multiplicity of infection of 3. After 30 min, the culture was diluted 1,000-fold into TPG2A at 35 C. At 0, 16, 21, and 26 min after the 1,000-fold dilution, samples were further diluted 10-fold into TPG2A at 42 C. Samples were removed at regular intervals and analyzed for intracellular phage.

Bacteriophage $\phi X174am3$ was supplied by R. L. Sinsheimer. This phage possesses an amber mutation in gene E (lysis), thus permitting observations on phage production in su^- hosts over long periods of time without the complication of host cell lysis. Bacteriophage $\phi Xahb$, which is a derivative of $\phi X174am3$, has been described in detail elsewhere (Primrose and Dowell, *in preparation*). Whereas $\phi X174$ replicates poorly at temperatures above 40 C (Hutchison, Ph.D. Thesis; Dowell and Dapper, *in preparation*), $\phi Xahb$ has a burst size of 107 at 42 C in an su^- host.

Other materials and methods not explained in the figure legends have been described by Primrose and Dowell (*in preparation*).

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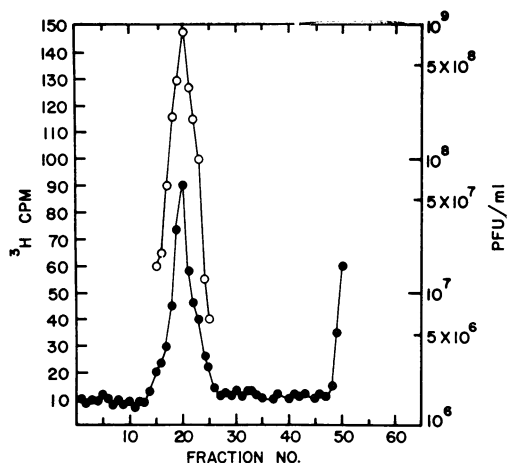


FIG. 3. Isolation of ^3H -labeled phage. Cells of *E. coli* t3 were grown in TPG2A to 3×10^8 per ml, centrifuged, and resuspended in starvation buffer containing 0.01 M Ca^{2+} and 0.009 M KCN . ϕXahb was added at a multiplicity of infection of 3. After 30 min, the culture was centrifuged and resuspended in TPG2A at 35 C. After 16 min of resuspension, a 20-ml sample was diluted into 40 ml of TPG2A at 42 C containing $10 \mu\text{Ci}$ of ^3H -thymine per ml as sole thymine source. Samples were removed at regular intervals for assay of intracellular phage and trichloroacetic acid-precipitable counts. After 50 min, a 20-ml sample was removed for isolation of labeled phage. The cells were concentrated by centrifugation and disrupted as previously described (11). The phage particles were eluted from the cell debris by incubation for 4 hr at 4 C with 1 ml of saturated sodium tetraborate. A 1-ml amount of the phage suspension was layered on a 30-ml 5 to 20% sucrose gradient containing 0.05 M borate and centrifuged for 3.5 hr at 24,000 rev/min in a Spinco SW25 rotor. Fractions (0.5 ml) were collected and assayed for radioactivity and phage infectivity.

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