Growth of a Capsid Mutant of Bacteriophage \( \phi X174 \) in a Temperature-Sensitive Strain of \textit{Escherichia coli}.

Christine C. Vito, Sandy B. Primrose and C. E. Dowell

Growth of a Capsid Mutant of Bacteriophage φX174 in a Temperature-Sensitive Strain of Escherichia coli

CHRISTINE C. VITO, SANDY B. PRIMROSE,1 AND C. E. DOWELL*

Department of Bacteriology, University of California, Davis, California 95616 and Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002*

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A capsid mutant of φX174 is capable of forming replicative form and synthesizing single strands at the restrictive temperature in a dnaB mutant of Escherichia coli. Under similar conditions, the wild-type bacteriophage is incapable of either step in viral synthesis.

In 1968 Steinberg and Denhardt demonstrated that in a dnaB mutant (18) of Escherichia coli, Bonhoeffer strain 7 (1), both replicative form (RF) formation and single-strand (SS) synthesis (phage growth) were inhibited at the restrictive temperature (16). Identical information was provided by Sinsheimer et al. in 1968 in a 15 TAU-bar strain of E. coli temperature sensitive for DNA synthesis (15). As far as we are aware, the genetic lesion in this latter strain has not been mapped, although on the basis of phenotype evidence we consider it likely to be a dnaB strain. In this paper we provide evidence that both blocks to φX174 replication can be relieved by a mutation in the φX174 capsid.

(This information is taken in part from a thesis submitted to the graduate school of the University of California, Davis, by S.B.P. and from a thesis to be submitted to the graduate school of the University of Massachusetts, Amherst, by C.C.V.)

MATERIALS AND METHODS

Bacteria and phage stocks. Bacteria and phage stocks used in this study are listed in Table 1.

Media and chemicals. KCT top and bottom agar (5), KCT broth (5), and TPG2A (8) have been described. The starvation buffer used was that of Denhardt and Sinsheimer (3). Borate-EDTA elution buffer (9) and NET buffer (2) have been described.

Chemicals. Cesium chloride (CsCl) and Sarkosyl (sodium lauryl sarcosinate) were purchased from K and K Laboratories. Chloramphenicol (CAP) was purchased from Calbiochem. Lysozyme LY (egg white) was purchased from Mann Laboratories, and protease was purchased from Sigma Chemical Co. The liquid scintillation fluid used to count samples from sucrose velocity gradients was Aquasol, purchased from New England Nuclear. Isotopically labeled compounds were also purchased from New England Nuclear.

Antisera inactivation. Preparation of antisera to disrupted φX174am3 particles and sera inactivation studies were previously reported (15). Antisera were diluted 1:10,000 in KCT. Phage was diluted in KCT to a concentration of 104/ml. Inactivation was carried out by mixing 0.9 ml of antisera with 0.1 ml of phage at 34 C for 5 min, with 0.1-ml samples being withdrawn at 1-min intervals and diluted 1:10,000 in cold KCT to stop inactivation. Surviving phage was measured by appropriately diluting and plating on the permissive host strain.

Heat inactivation. Phage, pre-equilibrated in saturated sodium tetraborate at 4 C, was added to saturated sodium tetraborate preheated to 56 C (pH of this solution is approximately 9.3). At various intervals, samples were withdrawn and diluted in cold sodium borate to stop the inactivation process. Samples were plated and assayed for PFUs.

One-step growth experiment and intracellular phage assay. Procedures have been described previously (3). In the case of phage replication at the restrictive temperature, cells were preincubated at the restrictive temperature for 10 min prior to addition of phage. Temperature shifts are as indicated in the legend.

Preparation of isotopically labeled bacteriophages. [3H]- and [14C]thymidine-labeled φX174 was prepared as follows. HF4704 was grown at 35 C to 3 × 108 cells/ml in TPG2A containing 2 μg of thymine per ml. Isotope (25 μCi) was added to 40 ml of cells 10 min prior to addition of phage. Phage was added at a multiplicity of infection of 3, and cells were incubated for 60 min at 35 C. The cells were harvested and resuspended in 1.5 ml of 0.033 M Tris (pH 8.1). One-tenth milliliter of lysozyme (2 mg/ml) and 0.15 ml of EDTA (0.8% in 0.033 M Tris) were added, and the mixture was shaken for 5 min. The cells were then frozen and thawed six times using a dry ice-acetone bath. A 2-ml amount of 0.05 M sodium borate was added to the lysate. After overnight incubation at 4 C, the lysate was centrifuged and the supernatant was collected. Phage was purified by CsCl centrifugation.

1Present address: University of Warwick, Coventry, CV4 7AL, England.
**Table 1. Bacteria and phage stocks used**

<table>
<thead>
<tr>
<th>Bacteria and phage stocks</th>
<th>Description</th>
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<tbody>
<tr>
<td>E. coli C suII</td>
<td>Standard host for φX174</td>
</tr>
<tr>
<td>HF 4714 suII*</td>
<td>Permissive host for φX174 amber mutants</td>
</tr>
<tr>
<td>HF 4704 suII*</td>
<td>Nonpermissive host for φX174 amber mutants</td>
</tr>
<tr>
<td>PRO501 dnaB</td>
<td>Derivative of Bonhoeffer strain 7 (1). Sensitivity to host range mutants of φX174 was induced by NTG mutagenesis according to the method of Tessman (17). The temperature-sensitive lesion has been found to lie in the dnaB gene (18).</td>
</tr>
<tr>
<td>φX174</td>
<td>Wild-type phage. Under conditions of high calcium ion concentration (10^{-2} M), this phage will infect PRO501.</td>
</tr>
<tr>
<td>φXam3</td>
<td>Lysis-defective mutant of φX174. Under conditions of high calcium ion concentration (10^{-2} M), this phage will infect PRO501.</td>
</tr>
<tr>
<td>φXahb</td>
<td>High-temperature-resistant and host range mutant of φXam3. This double mutant was isolated from φXam3 in two steps. φtam3, which was isolated for its ability to grow and produce large, clear plaques at the high temperature, was screened for its ability to plate on the non-temperature-sensitive parent of PRO501 at the high temperature. φtam3, as well as φXahb, exhibits altered thermal stability relative to φXam3 and is therefore assumed to be a capsid mutant. (C. C. Vito and C. E. Dowell, unpublished data). Under conditions of low calcium ion concentration (10^{-2} M), φXahb will infect PRO501: the efficiency of plating at 10^{-2} M calcium ion concentration is 100% in contrast to φXam3 or φX174, which is 0%.</td>
</tr>
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</table>

(see following) and dialyzed against 0.05 M sodium borate. The specific activity of H- and 14C-labeled purified phage was approximately 10^{4} counts/min per PFU.

2P-labeled phage was prepared as above with the following exceptions. E. coli C was grown to 3 × 10^{8} cells/ml in TPG2A at 35 C. 5P (5 mCi) (in water) was added to 40 ml of cells 10 min prior to addition of phage. The specific activity of 2P-labeled purified phage was approximately 10^{4} counts/min per PFU.

**Purification of H-, 14C-, and 32P-labeled bacteriophage.** The phage-containing supernatant was mixed with 0.625 g of CsCl per g of sample to yield a density of 1.44. Centrifugation was at 37,000 rpm for 36 h at 4 C in a SW50.1 rotor of a Spinco L2-65B ultracentrifuge. After centrifugation to equilibrium in CsCl, phage stocks were dialyzed and stored in 0.05 M sodium borate at 4 C.

**Infection of PRO501 at 42 C.** PRO501 was grown to 10^{8} cells/ml in KCT containing 10^{-2} M CaCl_{2} at 35 C. CAP was added to a final concentration of 150 μg/ml, and the cells were incubated for 10 min at 35 C. The culture was then shifted to 42 C.

The following procedures, including centrifugation, were carried out at 42 C. H-labeled φXahb or 32P-labeled φXam3 was added at a multiplicity of infection of 50, and the infection was allowed to proceed at 42 C. After 30 min, KCN was added to a final concentration of 10 mM, and the cells were harvested by centrifugation. The pellet was resuspended and washed three times in 3 ml of elution buffer containing 10 mM KCN and 150 μg of CAP per ml. The cells were then washed twice and resus-
Inactivation of phage by heat. The data in Fig. 1 show \( \Phi Xahb \) to be less stable under conditions of heat inactivation than \( \Phi Xam3 \). It is highly likely from these results that the mutations in \( \Phi Xahb \) result from an alteration(s) in a phage capsid protein(s) (14).

Inactivation of phage by antisera. The data in Fig. 2 reflect the percent surviving phage at each interval as compared to 100% active phage at time zero. Controls (not shown) following the rate of inactivation of \( \Phi Xam3 \) over a 20-min period show the rate to remain constant, indicating an excess of free antibody in solution. The data show that \( \Phi Xahb \) results from a change in the viral capsid. The data in Fig. 2 were reproducible in four independent experiments.

Parental RF formation in \( dnaB \) strain

![Fig. 1. Heat inactivation of whole particles of \( \Phi X174am3 \) and \( \Phi Xahb \) at 56 C. Inactivation is expressed as loss of PFUs per milliliter versus incubation time at 56 C. Symbols: \( \bullet \), \( \Phi X174am3 \); \( \square \), \( \Phi Xahb \).](http://jvi.asm.org/)

![Fig. 2. Antisera inactivation of whole particles of \( \Phi X174am3 \) and \( \Phi Xahb \) at 35 C. Inactivation is expressed as percent surviving phage versus time of incubation with antisera at 35 C. Symbols: \( \blacksquare \), \( \Phi X174am3 \); \( \bullet \), \( \Phi Xahb \).](http://jvi.asm.org/)
The results of experiments in which PRO501 was infected with $^{32}$P-labeled $\phi$Xahb and $^{32}$P-labeled $\phi$Xam3 at the permissive temperature are shown in Fig. 6 and 7, respectively.

**SS synthesis and phage production at 42 C.** If infected cells are shifted to 42 C before phage synthesis begins (1 min), no phage is produced (Fig. 8). However, if the shift is made after phage production has begun at 35 C, as much phage is produced as in an unshifted culture. To insure that phage production at 42 C did not result from the depletion of a pool of viral single strands made at 35 C and simply encapsidated at 42 C (an unlikely event since no pool of free single strands has been observed in $\phi$X174-infected cells), the culture was shifted 40 min postinfection to $^{3}H$-labeled thymine-containing media at 42 C. The DNA was extracted and layered on a sucrose gradient for analysis of intracellular DNA forms (Fig. 9). The analysis shows a large number of labeled single strands, a small peak of RF, and no reactivation of host cell DNA synthesis. That this DNA is actually encapsidated into mature phage particles is illustrated by the coincidence of radioactivity and infectivity in Fig. 10.
DISCUSSION

Capsid mutations in \( \phi Xahb \). It is evident from the heat and antisera inactivation studies that the \( ahb \) mutation(s) is a capsid mutation(s). Although we do not know the site(s) of the phage \( ahb \) mutations, we suspect that the H protein is involved (D. Bone et al., unpublished data). In support of these unpublished findings are the implications of the antisera inactivation studies, which are as follows. The fact that \( \phi Xahb \) reacts more slowly with respect to inactivation with antisera suggests that \( \phi Xahb \) has altered adsorptive properties. The adsorption specificity of \( \phi X174 \) is thought to be determined by the phage gene H product (S. M. Jazwinski, personal communication). Newbold and Sinsheimer have also shown that the gene H product is involved in the attachment of the phage to the bacterial receptor site (10). Therefore, the antisera inactivation studies reported here are consistent with the \( \phi Xahb \) mutation being in gene H. It is, of course, possible that a mutation in another coat protein could change the configuration of the spike and thus alter adsorption and antibody reactivity. Work is in progress to clarify this point.

Involvement of the phage capsid in RF formation and SS synthesis. Jazwinski et al. have shown that a M13 coat protein enters the cell with the M13 DNA; this is believed to occur with \( \phi X174 \) also (7). Doniger and Tessman have shown that a mutant of bacteriophage S13 with a temperature-sensitive lesion in gene H (spike protein) can make no RF DNA at the restrictive temperature (4). Both these results suggest that

![Fig. 6. \( \phi Xahb \) RF formation in PRO501 at 35 C. The direction of centrifugation is from right to left. Cells \( (8 \times 10^7) \) were infected with \( 32P \)-labeled \( \phi Xahb \) and treated as described in Material and Methods. The position of SS \( \phi X174 \) viral DNA marker is indicated by the arrow. Symbol: ■, \( 32P \)-labeled input \( \phi Xahb \).](http://jvi.asm.org/)

![Fig. 7. \( \phi Xam3 \) RF formation in PRO501 at 35 C. The direction of centrifugation is from right to left. Cells \( (8 \times 10^7) \) were infected with \( 32P \)-labeled \( \phi Xam3 \) and treated as described in Material and Methods. The position of SS \( \phi X174 \) viral DNA marker is indicated by the arrow. Symbol: ▲, \( 32P \)-labeled input \( \phi Xam3 \).](http://jvi.asm.org/)

![Fig. 8. PRO501 was grown in TPG2A to \( 10^7 \) cells/ml at 35 C. Cells were infected with \( \phi Xahb \) as previously described (13). At various times after infection portions of the infected culture were shifted to 42 C. All the assay points represent plaques present in the intracellular phage assay (3). Symbols: ●, 1-min shift; ○, 16-min shift; □, 18-min shift; ■, 22-min shift; ▲, no shift.](http://jvi.asm.org/)
a coat protein, possibly the gene H product, is involved in parental RF formation.

The data presented in this paper can be explained in the following manner. An altered ϕX174 gene H product allows RF formation to occur by (i) interacting with the host cell's altered dnaB product to form a functional complex, or (ii) allowing the incoming viral DNA to be recognized and replicated by an enzyme system previously incapable of doing so. If the gene H product remains associated with the replicating ϕX174 DNA during SS synthesis, the same arguments would pertain.

Since ϕXahb makes no mature phage particles upon infection at 42°C, a possible site for a replication block is in the synthesis of daughter RF DNA. Similar conclusions have been reached by Olsen et al. in their study of M13 (11).

Obviously, much more work needs to be done to understand the intimate relationship between phage and host proteins in the replication of small bacteriophage DNA.

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LITERATURE CITED


REPLICATION OF A ϕX174 CAPSID MUTANT


