Novel Replicative Properties of a Capsid Mutant of Bacteriophage φX174

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Received for publication 1 December 1975

A capsid mutant of bacteriophage φX174 demonstrates altered requirements for the conversion of viral single-stranded DNA to double-stranded replicative form DNA. In the presence of puromycin at 42°C, wild-type φX174 is unable to complete this replicative event, whereas φXahb is able to do so. Furthermore, in contrast to wild-type φX174, formation of φXahb parental replicative form DNA is sensitive to rifampin under certain experimental conditions. These data suggest that the mutant capsid proteins of φXahb influence the biosynthesis of φXahb complementary strand DNA.

Much of the data presently available suggest that, upon infection, interactions between phage and cellular components occur, which result in the expression of phage-specific processes. Phage-specific changes in the host replicative and transcriptional apparatus have been described in lambda (8, 9, P2 (30, 31), SPO1 (19), and T phage- (2, 16, 17, 27) infected cells.

Interesting observations, which point to the importance of phage-host interactions in replication, have also come from the study of the small, single-stranded DNA bacteriophages, φX174, S13, and M13. It has been found that capsid proteins are injected into the cell along with the phage DNA. These “pilot proteins” are thought to be involved in linking the phage DNA to a cellular replicative system (11, 12, 13, 22). Phage mutants, temperature-sensitive in these capsid proteins, are unable to produce replicative form (RF) DNA at the restrictive temperature (3, 11, 14). The successful conversion of single-stranded DNA to the double-stranded RF DNA is, at least partially, dependent upon the ability of capsid proteins to functionally interact with host components. It appears that the biological activity of such phage-host complexes may be dependent upon the “proper fit” of phage components with host components (2, 8, 9, 27, 30, 31).

As predicted by both the principle of proper fit and the pilot protein theory, we have isolated capsid mutants of φX174 that are able to grow in strains of Escherichia coli with structurally altered dna gene products (1, 10, 20, 33). One such capsid mutant, φXahb, was shown to synthesize both parental RF DNA and progeny viral DNA in the absence of functional dnaB protein (20, 33). The inability of wild-type φX174 to replicate under identical conditions is well documented (5, 26, 29, 33). We offered two alternative explanations of these observations (33). The nature of the φXahb capsid protein mutations may allow it to functionally interact with the mutant dnaB protein, and thus be replicated by the usual host biosynthetic apparatus. Or, alternatively, the nature of the φXahb capsid protein mutations may allow it to interact with host proteins not ordinarily involved in wild-type φX174 replication.

Because we were interested in pursuing the observation that φXahb has a mode of replication different than that of φX174, and because we felt that further experiments examining the ability of φXahb to replicate in host cells containing mutant dnaB proteins would not be useful in this regard, we designed the experiments presented in this report. We hoped that, by imposing different replicative restrictions upon both phages, circumstances might be found in which φXahb would again exhibit unique replicative abilities. We chose, therefore, to study phage replication in antibiotic-treated host cells. We reasoned that, in cells treated with antibiotic before phage infection, only the stable, long-lived cellular proteins would be available to the viral DNA template. If parental RF DNA formation was dependent upon unstable, short-lived cellular proteins, however, this replicative event might be inhibited in an antibiotic-treated host cell.

The data presented below show that, under certain experimental conditions, the requirements for φXahb parental RF DNA formation differ from those of φX174, suggesting that φXahb is able to interact with a new spectrum of host cell proteins.
(This information is taken in part from a thesis to be submitted to the graduate school of the University of Massachusetts, Amherst, by C. C. Vito.)

MATERIALS AND METHODS

Bacteria and phage stocks. E. coli C is the standard host of \( \phi X174 \). PR0501 dnaB is a temperature-sensitive strain of \( E. coli \) which grows normally at 35 C but not at 42 C; the isolation of this strain has been described previously (33). The kinetics of inhibition of DNA synthesis of PR0501 dnaB at the restrictive temperature are indistinguishable from the parent, Bonhoeffer strain 7. Incorporation of \(^{14}\)C thymidine into acid-precipitable material is reduced by greater than 99% after 15 min at 42 C. A spontaneous, temperature-resistant revertant of this strain was isolated at 42 C at a frequency of \( 1 \times 10^{-4} \). \( \phi X174am3 \) is a lysis-defective mutant of \( \phi X174 \) and is used in this report as the wild-type bacteriophage. \( \phi Xabh \) is a spontaneous, high-temperature resistant, host-range mutant of \( \phi X174am3 \) (33). This mutant was isolated from \( \phi X174am3 \) in two steps (33). \( \phi X6htam3 \), which was isolated for its ability to grow and produce large clear plaques at 42 C, was screened for its ability to plate at 42 C on the non-temperature-sensitive parent of PR0501. \( \phi X6htam3 \) and \( \phi Xabh \) exhibit altered thermal stability and altered antiserum inactivation relative to \( \phi X174am3 \), and are therefore considered to be capsid mutants (33).

Media and chemicals. Growth media, buffers, and other materials used in the experiments described in this paper have been described previously (33). Puromycin and rifampicin were purchased from Calbiochem.

Preparation and purification of \(^{3}\)H-, \(^{14}\)C-, and \(^{32}\)P-labeled bacteriophages. Labeling procedures and cesium chloride purification of radioactively labeled phage stocks have been described previously (33).

Parental RF DNA formation at 35 C. Cells were grown to \( 10^9 \) cell/ml in KCT broth containing \( 10^{-2} \) M CaCl\(_2\) at 35 C. Antibiotic was added, and the cells were incubated for 10 min at 35 C; puromycin or rifampicin was present at a final concentration of 90 \( \mu \)g/ml or 200 \( \mu \)g/ml, respectively. Radioactively labeled phage was added at a multiplicity of infection of 50, and the infection was allowed to proceed at 35 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 the appropriate antibiotic. The cells were then washed twice and resuspended in 3 ml of 0.1 M NET buffer, containing 10 mM KCN and the appropriate antibiotic. Lysozyme (0.035 ml) (4 mg/ml) was added to the cell suspension, and after 10 min, 200 \( \mu l \) of 10% Sarkosyl and 200 \( \mu l \) of Protease per ml (predigested, type VI, Sigma Chemical Co.) were mixed with the lysate by gentle rolling and allowed to mix for 2.5 h at 42 C.

Cesium chloride density gradient analysis of the Protease digest. The digest was mixed with 1.25 g of CsCl/g of sample to yield a density of 1.7 g/cc. Centrifugation was at 37,000 rpm for 40.5 h at 4 C in a SW50.1 rotor of the Beckman L2-65B ultracentrifuge. Four-drop fractions were collected from the bottom of the centrifuge tube, and the radioactivity of each fraction was determined in a Packard liquid scintillation spectrometer.

Parental RF DNA formation at 42 C. Cells were grown to \( 10^9 \) cell/ml in KCT broth containing \( 10^{-2} \) M CaCl\(_2\) at 35 C. Antibiotic was added, and the cells were incubated for 10 min at 35 C; puromycin or rifampicin was present at a final concentration of 90 \( \mu \)g/ml or 200 \( \mu \)g/ml, respectively. The culture was then shifted to 42 C and equilibrated for 15 min. The following manipulations, including centrifugation, were carried out at 42 C. Radioactively labeled phage 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 the appropriate antibiotic. The cells were then washed twice and resuspended in 3 ml of 0.1 M NET buffer, containing 10 mM KCN and the appropriate antibiotic. Lysozyme (0.035 ml) (4 mg/ml) was added to the cell suspension, and after 10 min, 200 \( \mu l \) of 10% Sarkosyl and 200 \( \mu l \) of Protease per ml (predigested, type VI, Sigma Chemical Co.) were mixed with the lysate by gentle rolling and allowed to mix for 2.5 h at 42 C.

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RESULTS

Effect of antibiotic treatment on host cells. As first observed by Nathans (18), the permeability of \( E. coli \) to puromycin is highly dependent upon the \( \text{Ca}^{2+} \) ion concentration of the growth media. Therefore, it was necessary to expose \( E. coli \) C to puromycin in the presence of \( 4 \times 10^{-3} \) M \( \text{Ca}^{2+} \) ion concentration (or lower) to achieve depressed levels of protein synthesis and inhibition of bacterial growth. The results of treatment of an exponentially growing culture of \( E. coli \) C with 90 \( \mu l \) of puromycin per ml are shown in Fig. 1A. For maximum phage adsorption to puromycin-treated cells, it was necessary to increase the \( \text{Ca}^{2+} \) ion concentration of the growth media to \( 10^{-2} \) M upon the addition of phage. In an experiment carried out under these conditions, we found that such an increase in the \( \text{Ca}^{2+} \) ion concentration did
not reverse the inhibitory effect of puromycin; data similar to those shown in Fig. 1A were obtained.

As previously observed (25), treatment of an exponentially growing culture of E. coli with 200 μg of rifampin per ml results in shutdown of bacterial RNA synthesis and inhibition of bacterial growth. The data shown in Fig. 1B were obtained upon exposure of E. coli C and PR0501 dnaB to 200 μg rifampin per ml.

Formation of parental RF DNA in the presence of puromycin. To test the effect of puromycin on parental RF DNA formation, E. coli C cells pretreated with 90 μg of puromycin per ml were infected with 32P-labeled φX174 or φXahlb. The conversion of single-stranded viral DNA to double-stranded RF DNA was examined by equilibrium centrifugation of the infected cell lysates in neutral cesium chloride density gradients.

Figures 2 and 3 show the results of such an analysis of cell lysates infected at 35°C. Almost all of the φX174 [32P]DNA (Fig. 2) and φXahlb [3H]DNA (Fig. 3) in each gradient banded at a position characteristic of RF DNA, indicating that efficient conversion of both viral DNAs to parental RF DNA occurred at 35°C. As confirmation, sucrose gradient sedimentation analysis of RF DNA from such a cesium chloride gradient showed a sedimentation velocity characteristic of RF I DNA molecules in a high salt sucrose gradient (33).

Because the mutant properties of φXahlb are more apparent at elevated temperatures at which it was selected (33), we examined parental RF DNA formation in the presence of puromycin also at 42°C. Experiments identical to those performed at 35°C were repeated at 42°C, and the results are shown in Fig. 4 and 5. Although the conversion of φX174 viral DNA to parental RF DNA has been shown to occur in the absence of host protein synthesis, we...
found that this conversion did not occur in the presence of puromycin at 42 °C (Fig. 4). In contrast, efficient conversion of φXahb viral DNA to parental RF DNA occurred at 42 °C in the presence of puromycin (Fig. 5).

Formation of parental RF DNA in the presence of rifampin. Similar experiments, testing the ability of φX174 and φXahb to form parental RF DNA in E. coli C in the presence of rifampin at both 35 and 42 °C, were performed. In four separate tubes, 32P-labeled φX174 or φXahb was added to equal aliquots of rifampin-treated E. coli C cells at both 35 and 42 °C. The fate of the input viral DNA was determined by equilibrium centrifugation of the infected cell lysates in neutral cesium chloride density gradients.

Under these conditions, there was no block in φX174- or φXahb-specific RF DNA formation at either temperature. The formation of φXahb parental RF DNA in the presence of rifampin at 42 °C is shown in Fig. 6. Data (not shown) similar to those in Fig. 6 were obtained in each of the other experiments. These data show that the block in φX174 parental RF DNA formation in the presence of puromycin at 42 °C in E. coli C does not occur with rifampin at 42 °C in E. coli C.
Recently, it was shown that a rifampin-sensitive mechanism is involved in the conversion of M13 single-stranded viral DNA to double-stranded parental RF DNA in E. coli (28). We observed previously that φXahb exhibits certain synthetic capacities more characteristic of bacteriophage M13 than of φX174, e.g., ability to form parental RF DNA in a thermosensitive dnaB mutant of E. coli (5, 28, 33). Therefore, we tested the possibility that, although φXahb parental RF DNA formation is rifampin-resistant in wild-type cells (Fig. 6), it is sensitive in a dnaB mutant.

\[3^{2}P\)-labeled φXahb was added to equal aliquots of rifampin-treated PR0501 dnaB cells at both 35 and 42°C. The conversion of φXahb viral DNA to parental RF DNA was examined by equilibrium centrifugation of the infected cell lysates in neutral cesium chloride density gradients. As shown in Fig. 7, the conversion of φXahb viral DNA to parental RF DNA is inhibited in the presence of rifampin in PR0501 dnaB at 42°C. At 35°C, at which temperature this dnaB mutation is not expressed, formation of φXahb parental RF DNA occurred, as expected, in the presence of rifampin (data not shown). Furthermore, φXahb has the ability to form parental RF DNA in the absence of rifampin at both 35 and 42°C in PR0501 dnaB, as shown previously (Fig. 3 and 6, reference 33). These data show that φXahb parental RF DNA formation is rifampin-sensitive at 42°C in the presence of a thermosensitive dnaB host cell protein, but is rifampin-resistant at 42°C in the presence of a wild-type dnaB host cell protein.

DISCUSSION

φXahb is a capsid mutant of bacteriophage φX174 which behaves very differently than the wild-type phage under certain experimental conditions. In the presence of puromycin in E. coli C at 42°C, φXahb demonstrated the ability to form parental RF DNA, although the formation of φX174 parental RF DNA was blocked under identical conditions. At the low temperature, however, both φXahb and φX174 formed parental RF DNA in the
presence of puromycin in E. coli C. These data suggest that φX174 parental RF DNA formation is dependent upon host cell functions that are unstable and need to be continuously expressed at the high temperature. In contrast, φXahb parental RF DNA formation is not dependent upon such unstable host cell functions at 42°C. We believe that the φXahb viral DNA template is active in the presence of host cell functions inactive with the φX174 viral DNA template. Perhaps the mutant capsid proteins of φXahb enable it to utilize an alternate replicative pathway involving stable host cell functions.

It has been shown in vivo that φX174 parental RF DNA formation requires the dnaB and dnaG host proteins (5, 13, 29, 33). It is possible that in the presence of puromycin at 42°C one or both of these proteins is labile, thus blocking φX174 parental RF DNA formation. It has been previously shown that φXahb parental RF DNA formation is independent of the dnaB protein in vivo (33), but not in vitro (35). Furthermore, we have shown that φXahb progeny single-strand DNA synthesis and phage production are independent of the dnaG protein in vivo (Vito and Dowell, manuscript in preparation). Since both the dnaB and dnaG proteins are indispensable for bacterial DNA synthesis in vivo, it would be interesting to determine if host cell DNA synthesis is shut down at 42°C in the presence of puromycin. Of course, it is possible that cellular proteins other than the dnaB and dnaG proteins are labile at 42°C in the presence of puromycin. It is known that at least 10 (23), possibly 11 (35), cellular proteins are required for the in vitro conversion of φX174 single-stranded viral DNA to the double-stranded RF DNA.

φXahb also behaves differently than wild-type φX174 in rifampin-treated host cells. We studied parental RF DNA formation in rifampin-treated PR0501 dnaB host cells to examine our hypothesis that φXahb replicated in the fashion of bacteriophage M13. At least two distinct replicative systems have been resolved with respect to the small, single-stranded DNA phages; one is representative of the mechanism by which M13 replicates (7, 15, 22), and the other is representative of the mechanism by which φX174 replicates (22, 23, 32, 35, 36). There are three instances in which the differences between these replicative mechanisms are most striking: (i and ii) M13 parental RF DNA formation occurs in the absence of functional dnaB (28) and dnaG (21) protein, whereas that of φX174 is very much dependent upon the dnaB (5, 26, 29, 33) and the dnaG (14) proteins; (iii) the conversion of M13 viral DNA to parental RF DNA is sensitive to rifampin (22, 28, 34), whereas that of φX174 is resistant (22, 24, 25). Knowing that φXahb differs from φX174 in that (i) it forms parental RF DNA in the presence of puromycin at 42°C in E. coli C and (ii) it forms parental RF DNA and progeny viral DNA in the absence of functional dnaB (33) and dnaG protein, respectively, we thought it possible that the φXahb mutations allow it to utilize the host replicative apparatus (4) ordinarily involved in M13 DNA synthesis. To test this, we chose rifampin as our probe, taking advantage of the differential sensitivity to rifampin exhibited by φX174 and M13. Under conditions in which φXahb behaves like M13, i.e., in the absence of functional dnaB protein in PR0501 dnaB at 42°C, the formation of φXahb parental RF DNA was inhibited by rifampin. In the absence of rifampin, however, φXahb parental RF DNA formation occurred in PR0501 dnaB at 42°C (33). Furthermore, in the presence or the absence (33) of rifampin at 35°C in PR0501 dnaB, φXahb parental RF DNA formation occurred. In view of these data and those which show that φXahb does form parental RF DNA in the presence of rifampin in wild-type E. coli at 42°C, it seems that the pattern of φXahb replication is dictated by the availability of host functions. (During the preparation of this manuscript, we became aware of the data of Dumas et al. which show that wild-type φX174 parental RF DNA formation is also rifampin-sensitive in the absence of functional dnaC protein [8]. These data support our notion that the pattern of phage replication is dictated by the availability of host functions.)

Both φX174 and φXahb form parental RF DNA in rifampin-treated wild-type E. coli at 42°C, yet in the presence of puromycin φX174 parental RF DNA formation is inhibited at 42°C in wild-type E. coli. Treatment of E. coli with either puromycin or rifampin, however, results in depressed levels of protein synthesis. Rifampin inhibits the initiation of mRNA molecules via its interaction with the beta subunit of RNA polymerase, whereas puromycin inhibits the elongation of nascent polypeptides via its interference with peptide bond formation. It is possible that mRNA specific for the "puromycin-labile" protein is stable long enough to account for the continued availability of this protein in rifampin-treated cells. In puromycin-treated cells, on the other hand, a labile protein would selectively disappear regardless of the stability of its mRNA. Although possible, we do not believe cells treated
with rifampin 25 min before phage infection contain such long-lived mRNA molecules. Conceivably, the presence of rifampin may somehow stabilize the "puromycin-labile" protein. There is no evidence, however, that rifampin interacts with any cellular protein other than RNA polymerase, and it is well known that wild-type φX174 parental RF DNA formation is independent of an RNA polymerase priming event in vivo and in vitro.

Yet another explanation of these data (which was suggested to us by a reviewer) is the following. It has been shown that, in the presence of rifampin, a factor is released from RNA polymerase III which ordinarily prevents RNA polymerase III from priming the φX174 viral template (37). Therefore, in the presence of rifampin at 42°C in E. coli C, wild-type φX174 DNA can be converted to RF DNA by RNA polymerase III, whereas in the presence of puromycin at 42°C in E. coli C wild-type φX174 DNA can not be converted to RF DNA by RNA polymerase III. This interpretation is consistent with the data summarized in Fig. 4 and 6. However, in view of the data which suggest that the RNA primer involved in φX174 RF DNA formation is catalyzed by the dnaG protein (22), not by RNA polymerase, we do not think this explanation likely. This could be tested by adding rifampin to puromycin-treated cells and determining whether or not RF DNA formation occurs under these conditions.

We conclude that dXahb is equipped, by virtue of its mutant capsid proteins, to interact with a DNA biosynthetic apparatus not ordinarily active with the wild-type φX174 template. These data are consistent with the suggestion that the capsid proteins of small bacteriophages serve a dual function in phage growth; first, as structural determinants in the phage particle, and second, as participants in phage DNA biosynthesis.

ACKNOWLEDGMENTS

We wish to thank Albe y M. Reiner for many valuable discussions and his help in the writing of this manuscript. This work was supported in part by a grant from the University of Massachusetts, Amherst (Univ. of Mass. RUC No. 752079).

LITERATURE CITED


