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Pleiotropic Effects of Mutants in Gene A of Bacteriophage φX174

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It has previously been established that the functional gene A product of φX174 is required for double-stranded DNA replication and that mutants in gene A affect the lysis of the host cell. We report here other alterations of normal phenotype for a subset of gene A mutants suggesting additional functions of gene A. Mutants in the subset failed to terminate cellular DNA synthesis and were unable to efficiently inactivate the colony-forming ability of the host. Two mutants in a second group retained the ability to kill the infected cell, although only one of these mutants efficiently terminated cellular DNA synthesis. Normal termination of cellular DNA synthesis did not occur by the production of random multiple breaks in the DNA, although it may have occurred by the selective production of breaks in newly synthesized DNA. It has previously been shown that two protein products are produced from the gene A region, the smaller of which is a C-terminal fragment of the larger. The separate phenotypes reported here for the two groups of mutants in gene A are consistent with separate functions for the two gene products previously reported.

The life cycle of φX174 can be divided into three distinct phases. They are: conversion of the infecting single-stranded (SS) genome to the parental double-stranded replicative form (RF); replication of progeny RF molecules; and replication of SS DNA, maturation of virus particles, and lysis of the host cell. At the interface between the second and third phase, replication of RF DNA and cellular DNA are terminated (12). Termination of RF and cellular DNA replication requires new protein synthesis and hence is believed to be carried out by viral specified proteins (7, 13). However, the termination of cellular and RF replication has not been correlated with a specific viral gene product.

The gene A product of φX174 is required for progeny RF replication (8). Gene A mutant infections have also been reported to lyse later than wild-type infections (11). Two protein products of gene A have been described, the smaller one being a C-terminal fragment of the larger (9). The smaller fragment of 35,000 molecular weight arises from an internal initiator for transcription or translation and hence could be a functional product (9). The larger product of 65,000 molecular weight has been shown to carry site-specific endonuclease activity, and a role has been suggested for it in SS replication as well as RF replication (3).

In this report, several gene A mutants of φX174 were used in studies designed to determine the role of gene A in termination of cellular DNA synthesis and in cell killing after φX174 infection.

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MATERIALS AND METHODS

E. coli strains. *E. coli* HF 4704B (Su^-, Hcr^-, Thy^-, Sm^r) is a single-step variant of *E. coli* HF 4704 (Su^-, Hcr^-, Thy^-) previously described (7). The variant is resistant to 200 μg of streptomycin per ml and was obtained from T. Schwartz. Sm^r host cells are less permissive for some amber bacteriophage mutants (5). *E. coli* WWU $Su2_{oc}$ was used as the permissive host for amber mutants (10).

Phage strains. φX174 mutants *am*8A, *am*82, *am*84, and *am*85 (gene A); *am*3 (gene E); *am*10 (gene D); and *am*9 (gene G) have been previously described (1).

Media. TPGA, borate dilution fluid, and starvation buffer have been described (2). Lysis mixtures contained 0.2 mg of lysozyme per ml and 10^{-2} M EDTA. Lysis mixtures were prepared in either 0.2 M Trizma base (pH 8.1) or in the culture fluid. Dialysis solutions contained 0.01 M Trizma base (pH 8.1), 0.1 M NaCl, and 2.10^{-3} M EDTA.

Reagents. Mitomycin C and chloramphenicol were obtained from Sigma. Pronase (B grade) was obtained from Calbiochem; [^{3}H]thymidine (20 or 50 Ci/mmol) and [^{14}C]thymidine (55 mCi/mmol) were

obtained from New England Nuclear Corp. Sarkosyl (NL-30) was a gift of the Geigy Chemical Corp.

Growth of host cells. *E. coli* HF 4704B was grown with aeration in TPGA supplemented with 4 μg of thymidine and 50 μg of deoxyadenosine per ml. Where DNA synthesis was measured, the proportions of unlabeled and [^{3}H]thymidine were adjusted as appropriate but always to give 2 μg or more of total thymidine per ml.

Centrifugation procedure. Velocity sedimentation was in a Beckman SW50.1 rotor in 5 to 20% sucrose gradients. Sample size was either 100 or 200 μl. Some gradients contained a bottom pad of CsCl (p = 1.8) made in 20% sucrose. The sucrose solutions contained 0.01 M Trizma base (pH 8.0), 1.0 M NaCl, 0.05% Sarkosyl, and 10^{-2} M EDTA. Alkaline sucrose gradients were adjusted to pH 12.6 with solid NaOH.

Assay of radioactivity. Samples up to 100 μl were spotted on 22-mm Whatman 3MM filter disks and immediately added to ice-cold 5% trichloroacetic acid. The filters were batch processed three times in trichloroacetic acid and one time in acetone (15 min each) and separated on a paper towel to dry. Filters were counted in 10 ml of a toluene-Omnifluor cocktail (New England Nuclear Corp.) in a Beckman LS-250 liquid scintillation spectrometer. Crossover corrections for both ^{3}H and ^{14}C spillover were made where appropriate.

Gentle extraction of cellular DNA. Infected cells were removed to an ice bath, and lysozyme (0.2 mg/ml) and EDTA (0.01 M) were added. After incubation at 0 C for 30 min, Sarkosyl was added to 0.5%. Mixing was carried out on a Vortex mixer for 30 s at the lowest setting commensurate with efficient mixing. Lysates were warmed to 30 C for 5 min and frozen until assay. Freshly thawed lysates were again vortexed with marker DNA prior to sedimentation. Samples were applied to the sucrose gradients with a 1.0-ml Falcon plastic pipette. Cellular DNA prepared in this manner gave reproducible velocity sedimentation profiles with a maximum S value of about 75.

Normal extraction of phage DNA. Infected cells were chilled, pelleted, and suspended in Tris lysis mix. After incubation at 0 C for 30 min, Sarkosyl (0.5%) and Pronase (500 mg/ml) were added. The lysates were warmed to 46 C, incubated 12 to 16 h, and frozen until assayed.

Inhibition of cellular DNA by mitromycin C. To a growing culture of cells, mitromycin C was added to make 50 μg/ml. The culture was incubated at growth temperature for 30 min without aeration. The cells were then filtered onto a membrane filter (Millipore Corp.; 0.45-μm pore size), washed, and suspended in prewarmed medium without the drug. Care was taken to protect the cells from light after addition of the drug.

Growth and purification of isotopically labeled phage. *E. coli* WWU $Su2_{oc}$ cells were grown in supplemented TPGA medium to 10^8 cells/ml. The culture was filtered (Millipore, 0.45 μm) and suspended to give 5×10^8 cells/ml. Aliquots were infected with either *am*3 or *am*82 at a multiplicity of infection (MOI) of 5. [^{3}H]thymidine was added at 10 min postinfection to give a specific activity of 3.9 Ci/mmol. Cell lysis was aided by the addition of 0.02 M EDTA. Lysates were clarified by low-speed centrifugation.

Samples of 2.0 ml were purified by velocity sedimentation through 5 to 20% sucrose gradients (27,000 rpm, 200 min, 10 C, Beckman SW27 rotor, no Sarkosyl in sucrose). Fractions corresponding to the phage band were pooled, dialyzed, and frozen. The specific activity of the purified phage was about 4×10^{-5} dpm/PFU.

Parental RF formation by am3 and am82. *E. coli* HF 4704B was grown to 10^8 cells/ml, filtered (Millipore, 0.45 μm), and suspended in the same medium plus 150 μg of chloramphenicol per ml. The culture was split into two parts, incubated for 5 min at 37 C, and infected with either ^{3}H-labeled *am*3 or ^{3}H-lalabeled *am*82 phage at an MOI of about 3. After incubation for 15 min with aeration, the culture was chilled, pelleted, and washed three times with chilled borate dilution fluid containing 10^{-2} M EDTA and 150 μg of chloramphenicol per ml. The cells were pelleted again and suspended in 0.1 volume of lysis mixture containing 150 μg of chloramphenicol per ml. RF DNA was extracted with Sarkosyl-Pronase as described above.

Preparation of figures. All figures in this report were machine drawn using a Hewlett-Packard 9820A computer and 9862A plotter.

RESULTS

Inability of am82 and am8A to terminate host DNA synthesis. Stone (13) had previously reported that a gene A mutant of φX174, *am*8, terminated host DNA synthesis as efficiently as mutants in other genes. The original *am*8 mutant is a double mutant containing both amber and temperature-sensitive (*ts*) mutations. A revertant was isolated from *am*8 which retained the amber locus but was no longer *ts*, called *am*8A (F. D. Funk and R. L. Sinsheimer, unpublished data). In contrast to Stone's original observations, we found that *am*8A attenuates but does not completely terminate cellular DNA synthesis. Another mutant in gene A, *am*82, may be completely unable to terminate host DNA replication. These observations are represented in Fig. 1. Host DNA replication is normally shut off at 15 to 20 min after infection (7, 13). To make the assay more sensitive, [^{3}H]thymidine was added to the infected host cells at 25 min after infection, a time when termination of DNA replication in the positive control is complete. The slope for the *am*9 infection in Fig. 1 is less than 1% of the initial slope for the uninfected control. Part of the inhibition of DNA synthesis in the *am*9 infection is due to cell lysis, which began by 25 min and was essentially complete by 50 min. In contrast to the control, the *am*82 and *am*8A infections showed linear kinetics of DNA synthesis. The *am*8 infection consistently displayed a rate of DNA synthesis averaging about 50% that of *am*82

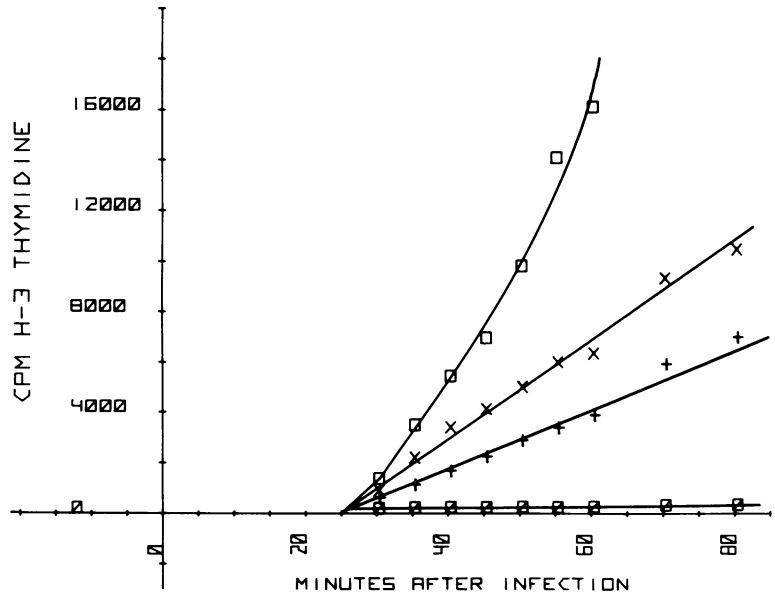

FIG. 1. *Kinetics of cellular DNA synthesis in φX174-infected cells. E. coli HF 4704B was grown to 10^8 cells/ml, divided into four aliquots of 3 ml each, and infected at an MOI of 5. At 25 min postinfection, [^{3}H]thymidine was added (final specific activity, 1.1 Ci/mmol). Aliquots (50 μl) were removed as a function of time and assayed for acid-precipitable radioactivity. Symbols: □, uninfected; ×, am82; +, am8A; ⊠, am9.*

(65% in Fig. 1). Neither *am*82 nor *am*8A efficiently terminated host DNA synthesis.

Partial and complete termination of host DNA replication by am84 and am85. The kinetics of host DNA replication in cells infected with *am*84 and *am*85 are shown in Fig. 2. Infection with *am*84 appears similar to *am*82 except that the rate of DNA synthesis declined until about 40 min after infection, whereupon it became linear. In contrast, infection with *am*85 showed a complete inhibition of cellular DNA synthesis. The termination of cellular DNA replication by *am*85 was not due to cell lysis. Qualitatively, none of the four gene A mutants tested showed significant lysis by 60 min postinfection. Hence at least one mutant, *am*85, retains the ability to terminate host DNA replication.

Size distribution of cellular DNA made after infection with am82. To analyze the DNA made in these cultures, samples were harvested at 50 min postinfection. The DNA was gently extracted so as to minimize shear and also to maintain a constant amount of shear from experiment to experiment. Lysates prepared from uninfected and *am*82-infected cultures were sedimented through neutral sucrose gradients (Fig. 3). DNA from uninfected cells sedimented as a broad band having a hypersharp leading edge. The material in the leading edge had an *S* value of about 75. Very little DNA sedimented in the region of the RFI marker region (21*S*) at fraction 35. The trailing material appeared in some experiments and not in others. The trailing material was cell DNA and not viral DNA since *am*82 produced no progeny viral DNA (see Fig. 7A). Hence the DNA replicated late in an *am*82 infection is host DNA.

Size distribution of cellular DNA made after infection with am84. The size distribution of host DNA replicated after infection with *am*84 was unique among the mutants studied (Fig. 4). Whereas *am*82 infections sometimes demonstrated a small amount of DNA sedimenting with the RF marker, *am*84 infections consistently showed about 40% of the radioactivity recovered in this region. Even though this 20*S* band of material seemed broad compared to the RF marker and hence was probably not homogeneous, the possibility remained that it represented progeny RF DNA. However, velocity sedimentation profiles of *am*84 infections where cellular DNA synthesis was inhibited by mitomycin C showed almost no labeled RF molecules (data not shown) and were identical to the profile shown in Fig. 7A for *am*82. Hence *am*84 does not produce progeny viral DNA, and the 20*S* material must be fragmented cellular DNA.

Mechanism of termination of host DNA replication. Having observed that one mutant

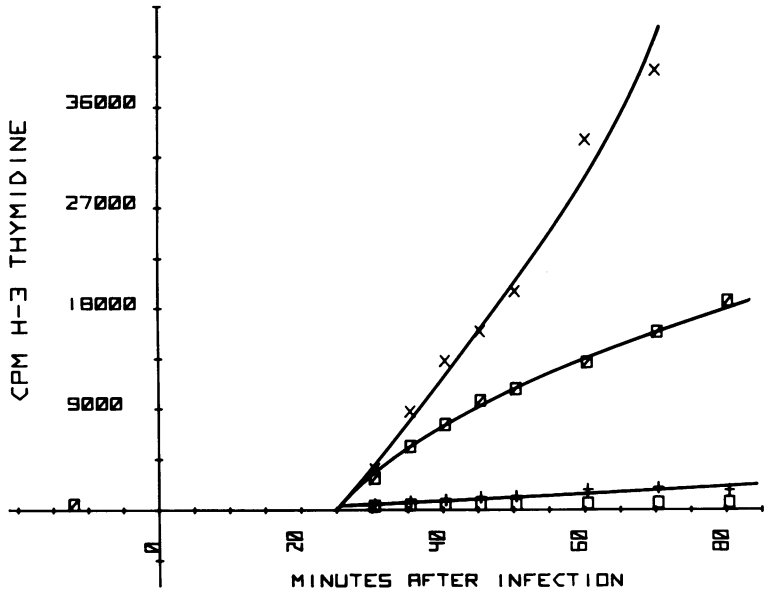

FIG. 2. *Kinetics of cellular DNA synthesis in φX174-infected cells. Experiment was conducted as described in the legend to Fig. 1 except [³H]thymidine was added at a specific activity of 2.3 Ci/mmol. Symbols: ×, uninfected; ⊠, am84; +, am85; □, am9.*

of gene A fragmented part of the host DNA synthesized from 25 to 50 min after infection, an experiment was designed to test whether fragmentation represented the general mechanism by which cellular DNA replication was terminated. *E. coli* HF 4074B was prelabeled with [^{14}C]thymidine for two generations prior to infection. At time zero, the culture was split. Each part was infected at an MOI of 5 with either *am*84, *am*82, or *am*9. One part was left uninfected. The [^{14}C]thymidine remained in the medium. At 20 min after infection, the cultures were lysed in an alkaline lysis mix (1.0 M NaCl-10^{-2} M EDTA-0.5% Sarkosyl, pH 13). All samples were gently manipulated to avoid shearing the DNA. The DNA size distribution was determined by velocity sedimentation through alkaline sucrose gradients. The radioactivity profiles of all four samples were virtually identical (data not shown). The DNA sedimented as a broad band with an average *S* value of 50 to 60. The *am*9 profile contained a small band of material sedimenting near the top of the gradient and representing about 4% of the total radioactivity. This is about the amount one would expect from the progeny RF made in the *am*9 infection. Hence φX174-induced termination of cellular DNA replication does not occur by widespread SS or double-strand breaks produced in the cellular DNA.

Cell killing by gene A mutants. Some gene A mutants killed nonpermissive host cells very inefficiently. At an input MOI of 5, 40 to 80% of *E. coli* HF 40704B cells survived an *am*82 infection. An interesting hypothesis arising from this observation was that all of the DNA replication sites might have to be occupied by RF molecules for the infecting virus to kill the host cell. The data presented in Fig. 5 demonstrate that this is not the case. The positive control cells, infected with the *am*9 mutant in gene G, were killed with an efficiency of nearly 1.0/PFU. At an input MOI of 7.5, the cell survival level observed (0.001) is equivalent to an effective MOI of 6.9 as calculated using the Poisson distribution function. In contrast, 40% of the cells survived an *am*82 infection at an input MOI of 10. If a threshold MOI were required before any cells were killed, the survival curve would show a shoulder typical of "multitarget" inactivation. No such shoulder was observed at input multiplicities up to 20. Hence there seems to be a constant probability that any infectious *am*82 virion will kill the cell. That probability, averaged over many experiments, is about 0.1. Equivalent results were obtained with *am*8A.

In contrast, the other two mutants studied, *am*84 and *am*85, killed infected cells with much greater efficiency. Averaging only two measurements, *am*84 had a probability of 0.5 and *am*85 had a probability of 0.7/PFU of killing the host cell. Eclipse of input phage was always greater than 95%. Under permissive conditions the *am*82 mutant killed the amber-suppressing host, *E. coli* HF 4714, with normal efficiency (data not shown).

Formation of parental RF by am82. *am82* neither efficiently killed the host cell nor shut off host DNA replication. Hence there was concern that expression of phage genes might not be occurring because of an inability to form the parental RF from infecting SS DNA. Stocks of *am3* (gene E) and *am82* were prepared which were lightly labeled with [^{3}H]thymidine. After infection of *E. coli* HF 4704B (see above), the conversion of the infecting SS to the double-stranded form was assayed by velocity sedimentation (Fig. 6). SS DNA sedimented about seven fractions ahead of the RFI marker DNA or about where the shoulder is seen in Fig. 6B (fraction 18). Nearly all (72 to 77%) of the labeled viral DNA which penetrates the host cell was converted to RF molecules for both mutant infections. Overall, about 30% of the radioactivity in the virus inoculum was found associated with RF molecules. Hence parental SS DNA in an *am82* infection is efficiently converted to the double-stranded form. If gene expression for

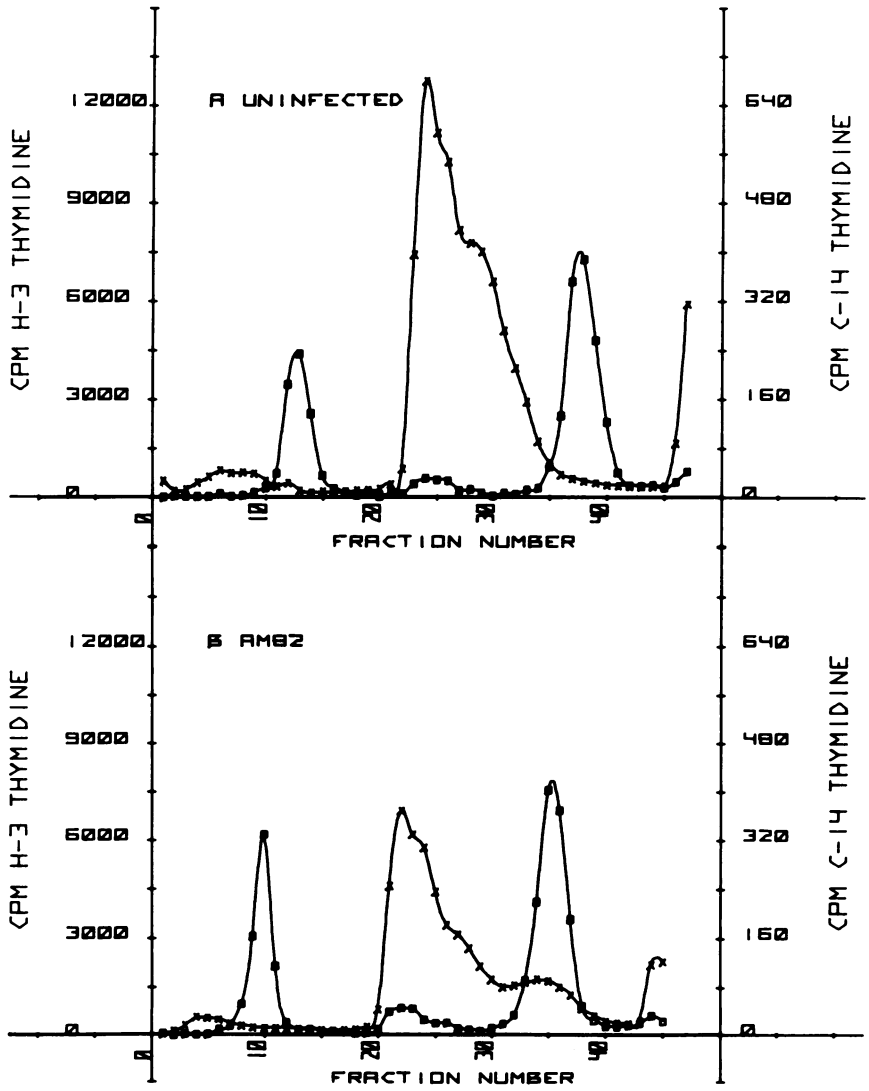

FIG. 3. *Size analysis of cellular DNA after infection with am82. Velocity sedimentation was in 5 to 20% neutral sucrose, 0.5-ml pad of CsCl (ρ = 1.8) in 20% sucrose. From an experiment similar to that described in the legend to Fig. 1, except that the MOI was 8 and [^{3}H]thymidine final specific activity was 5.2 Ci/mmol. Samples were harvested at 50 min postinfection and diluted twofold in lysis mix. Markers are φX174 virions at fraction 13 (114S) and RFI DNA at fraction 38 (21S). Sedimentation is from right to left. Symbols: ×, ^{3}H; ■, ^{14}C.*

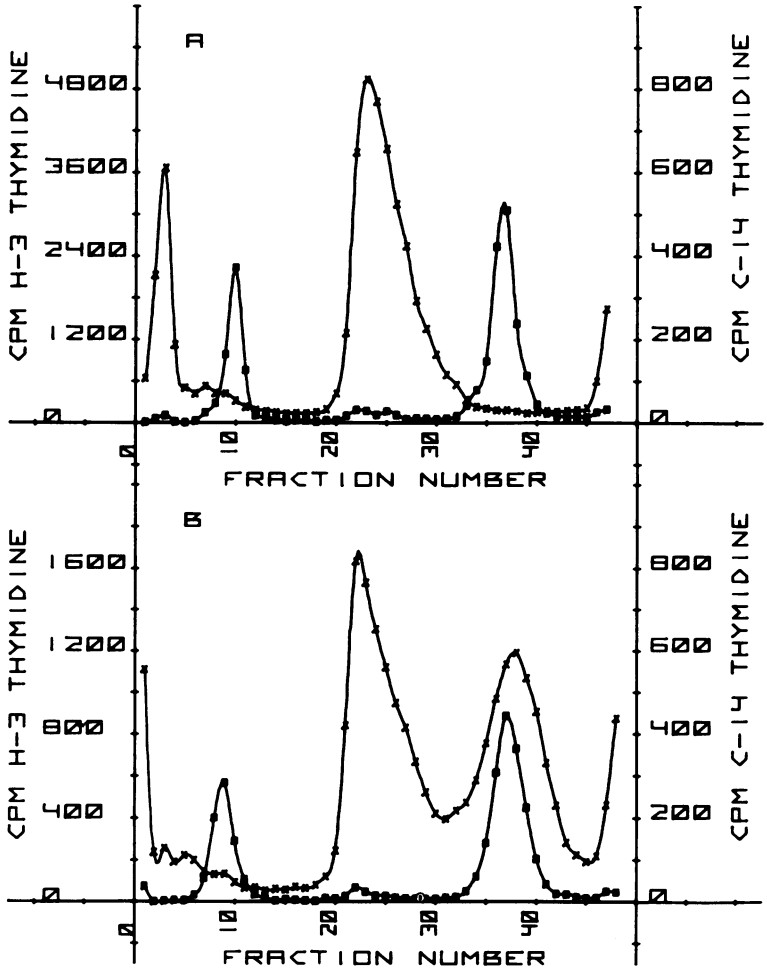

FIG. 4. *Size analysis of cellular DNA after infection with am84. Samples were taken at 55 min postinfection from experiment shown in Fig. 2. Centrifugation procedure was as described in the legend to Fig. 2 except samples were not diluted before lysis. Markers are φX174 virions (114S) and RFI DNA (21S). (A) Uninfected control; (B) am84 infection. Symbols:* ×, ^3H; ■, ^{14}C.

these mutants is blocked, it is not due to the inability to synthesize double-stranded parental RF.

Progeny RF synthesis by am82. Mutants in gene A are not efficiently rescued in a complementation test (11). Hence if mutants in two separate genes showed asymmetric complementation, they would be incorrectly assigned to the same gene. This possibility was tested by checking the phenotype characteristic of gene A mutants, the inability to replicate progeny RF DNA. Host DNA synthesis in the test cells was inhibited by the drug mitomycin C. Treated cells were infected and incubated in the presence of [^{3}H]thymidine for 25 min. After lysis and digestion with Pronase, the lysates were analyzed by velocity sedimentation (Fig. 7). The positive control, an *am*10 (gene D) infection shown in Fig. 7B, made a normal complement of progeny RF molecules. In contrast, the *am*82 infection had only about 2% as much labeled material sedimenting in the RF region. A small amount of radioactivity in the RF region would be expected due to synthesis of the complementary strand of the parental RF. Hence *am*82 is unable to produce progeny RF molecules consistent with its assignment to gene A. Identical results were obtained with *am*84.

DISCUSSION

Stone had previously reported (13) that *am*8 in gene A of φX174 efficiently terminated host DNA synthesis at a temperature of 40 C. We have no solid rationale for the discrepancy be-

tween Stone's results and those reported here for *am*8A. As previously mentioned, the original *am*8 mutant contained both a temperature-sensitive and an amber mutation. However, it seems unlikely that a second temperature-sensitive mutation could restore the ability to terminate host DNA replication. Results with *am*8A at 41 C were equivalent to those obtained at 37 C.

Data gathered using the mutants *am*82, *am*8A, *am*84, and *am*85 suggest that gene A mutants may be divided into two classes, each of which produces a separable phenotype. The characteristics assayed include the ability to terminate cellular DNA synthesis, the ability to kill the infected cell, and the ability to fragment cellular DNA. The mutants *am*82 and *am*8A are grouped in one class, and *am*84 and *am*85 make up the second class. The mutants *am*82 and *am*8A fail to terminate cellular DNA synthesis. These mutants also display an efficiency of about 0.1/PFU of killing the infected cell. No fragmentation of cellular DNA was observed with either mutant.

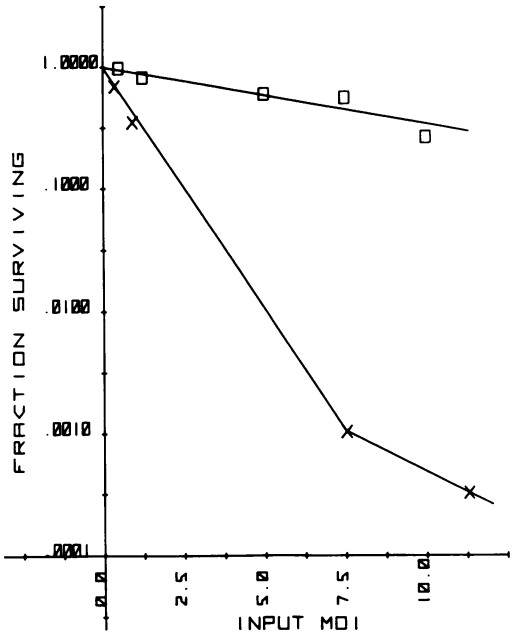

FIG. 5. *Cell killing after infection with mutants of φX174. E. coli HF 4704 B was grown in TPGA. At 10^8 cells/ml, the culture was removed to an ice bath and split into 2-ml aliquots. Each aliquot was infected with either am82 or am9 or not infected. The cultures were warmed to 37 C, incubated 15 min with aeration, and returned to an ice bath. Dilutions were made in chilled starvation buffer, and samples were assayed for colony-forming ability on tryptone agar plates. Symbols: □, am82; ×, am9.*

Although *am*84 and *am*85 are grouped together, their phenotypes are not identical. Infection with *am*85 results in nearly complete termination of cellular DNA synthesis by 20 min postinfection. Attenuation of cellular DNA synthesis in an *am*84 infection does not begin until about 30 min postinfection, and only a partial termination is achieved. Both *am*84 and *am*85 kill infected cells with a significantly higher efficiency, about 0.5 to 0.7 per infecting particle. In an *am*84 infection, about 40% of the cellular DNA made from 25 to 50 min is fragmented into pieces of about 20*S* compared to pieces of about 50 to 60*S* in the control. No significant differences in the size distribution could be detected when the samples were sedimented under neutral or alkaline conditions. These data suggest that a portion of the cellular DNA is fragmented late in an *am*84 infection. In addition, since the length distributions were similar under normal and denaturing conditions, the fragmentation did not occur by the random production of SS breaks in newly synthesized DNA. The observation that fragmentation occurs is very interesting in view of the endonuclease function assigned to the larger gene A product. Breakdown of cellular DNA in an *am*85 infection was not assayed.

It should be emphasized that no fragmentation of preexisting or newly synthesized cellular DNA was detected in cells infected with *am*9, *am*82, or *am*84 when assayed at 20 min postinfection. During this time period, the *am*9 infection had efficiently shut down cellular DNA synthesis. Hence φX174-mediated termination of cellular DNA synthesis is not due to widespread, random, SS, or double-strand breaks produced in the DNA. This data does not preclude the possibility that cellular DNA synthesis is normally shut off by a selective nuclease attack in a small portion of the DNA, perhaps at the replication fork.

For the *am*82 and *am*8A mutant infections in which host DNA replication is not terminated, the rate of synthesis is linear over the 25- to 80-min postinfection period studied. Linear DNA synthesis suggests that the infected cells are not accumulating functional DNA templates, upon which reinitiation of synthesis may occur. The rate of DNA synthesis for *am*82 infections is consistent with the rate one would expect from the uninfected control at 15 to 20 min postinfection. Hence the partial attenuation of DNA synthesis for *am*82 infections is probably due to the inability to initiate new rounds of synthesis rather than a decline in the rate of synthesis per replication fork. It should be emphasized that the apparent inability to initiate

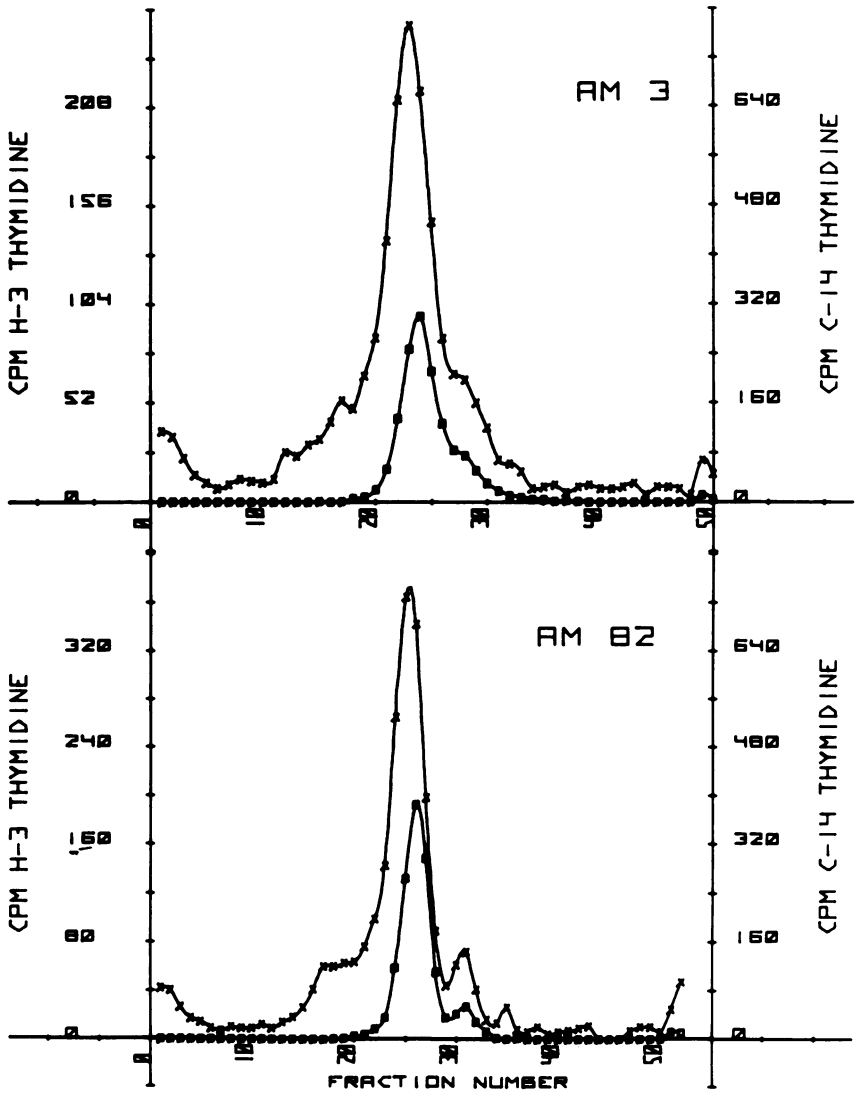

FIG. 6. *Conversion of am3 and am82 parental SS DNA to parental RF. Velocity sedimentation was in neutral 5 to 20% sucrose gradients of lysates of cultures previously infected with purified virions labeled with [^{3}H]thymidine (SW50.1 rotor, 45,000 rpm, 4 h, 5 C). See Materials and Methods for experimental details. Sedimentation is from right to left. Marker DNA is ^{14}C-labeled RFI. Symbols: ×, ^{3}H; ■, ^{14}C.*

new rounds of DNA replication may or may not be linked to the mechanism of termination of cellular DNA synthesis. It may have nothing to do with a gene A function, since the inability to initiate new rounds of host DNA synthesis would be masked in a wild-type infection, where host DNA synthesis is completely inhibited. The phenomenon must be transient in nature, since the *am*82 and *am*8A mutants demonstrate a probability of only 0.1 per infectious particle of killing the infected cell.

Linney and Hayashi (9) have shown that two products are produced from gene A. The smaller product of 35,000 molecular weight is identical to the C-terminal portion of the larger 65,000-molecular-weight product. In addition they have demonstrated that the smaller protein is not a cleavage product of the larger. The differential ability of the gene A mutants studied in this report to terminate cellular DNA replication and to kill the host cell suggests that both products are functional and that the functions are not identical. No direct evidence in terms of map position or gene A products produced is available for these mutants to indicate which pair might be responsible for elimi-

nation of the smaller 35,000-molecular-weight product. Endonuclease activity has been detected in the 65,000-molecular-weight product, and this activity is implicated in progeny RF synthesis (3). If both gene A products are functional, then by inference the 35,000-molecular-weight product would be implicated in cell killing and termination of cellular DNA synthesis, since mutations that eliminate the smaller product also eliminate the larger one (9).

In view of the pleiotropic effects of these mutants, a likely hypothesis is that the smaller gene A product affects expression of late phage functions. It could do this by controlling late expression in some specific fashion. A second plausible hypothesis is that the smaller product, in a nonfunctional state, binds strongly to RF DNA and nonspecifically inhibits phage expression. Incomplete expression of late phage functions is not due to a lack of parental RF DNA, since *am*82 parental RF is efficiently formed. Neither is the lack of expression entirely due to a single parental RF upon which to transcribe mRNA, since *am*84 partially termi-

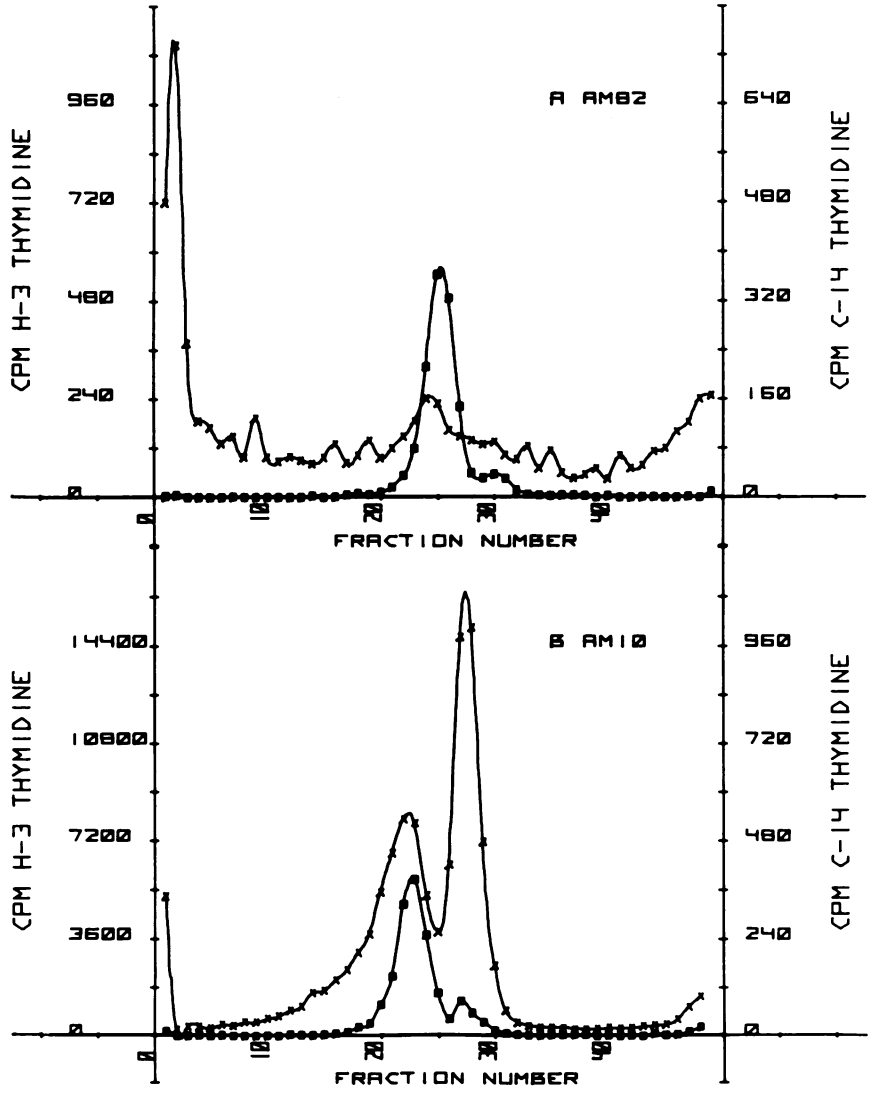

FIG. 7. *Progeny RF formation by am82 and am10. Cultures of E. coli HF 4704, pretreated with mitomycin C, were infected with either am82 or am10 in the presence of [^{3}H]thymidine (specific activity, 9.8 Ci/mmol; MOI, 5). Lysates were sedimented as described in the legend to Fig. 4. Marker DNA is ^{14}C-labeled RFI. Symbols: ×, ^{3}H; ■, ^{14}C.*

nates DNA replication and efficiently kills its host while making no or very few progeny RF molecules.

The inability of *am*82 or *am*8A to shut off cell DNA replication is not due to a polarity effect from the amber mutations. Essentially equivalent results were obtained with a newly isolated temperature-sensitive mutant, *am*3,*ts*82A (genes E, A). Cellular DNA synthesis is shut off by *am*3,*ts*82A under permissive conditions at 30 but not at 41 C. The control infection, *am*3,*ts*γ (genes E, G), attenuated cell DNA synthesis greater than 99% at 41 C (D. Snover and F. D. Funk, unpublished data).

ACKNOWLEDGMENTS

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ADDENDUM

Since this manuscript was submitted, a report containing similar data has been published by D. Martin and N. Godson (Biochem. Biophys. Res. Commun. 65:323-330, 1975). They have correlated the presence of the smaller 35,000-molecular-weight product of gene A with termination of cellular DNA synthesis.

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