Bacteriophage T4 *unf* (= *alc*) Gene Function Is Required for Late Replication in the Presence of Plasmid pR386

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The bacteriophage T4 *unf* gene, known to be involved in the arrest of transcription from cytosine-containing DNA, is unessential except in *Escherichia coli* strains containing plasmid pR386. Comparative genetic and biochemical analyses of parameters of *unf*⁺ and *unf*⁻ phage growth in host cells isogenic except for the presence or absence of plasmid pR386 have shown that *unf* gene function is required for late phage DNA synthesis in the presence of the plasmid. Shutoff of host DNA, RNA, and protein syntheses, degradation of host DNA, adsorption, injection, and early phage DNA, RNA, and protein syntheses all occurred with normal or near-normal kinetics in *unf*⁻ infections, even in the presence of the plasmid. The switch from early to late protein synthesis occurred in plasmid pR386-containing cells infected with *unf*⁺ or *unf*⁻ phage. However, this switchover was slow in both cases and may be slower in *unf*⁻ infections than in *unf*⁺ infections. Net incorporation of [³H]thymidine terminated at about 30 min after infection of pR386-containing cells with *unf*⁻ phage at 30°C. Alkaline sucrose gradient studies of the intracellular pools of replicative DNA in *unf*-infected plasmid pR386-containing cells indicated that this DNA is not detectably nicked or cleaved at the time that DNA synthesis aborts. The addition of chloramphenicol subsequent to early enzyme synthesis prevented the arrest of DNA synthesis in plasmid-containing cells infected with *unf*⁻ phage.

Bacteriophage T4 is known to induce the "unfolding" of the folded genome of the host cell within 5 min after infection (38). This unfolding is not induced by phage ghosts or in the presence of chloramphenicol (32). T4 mutants that fail to induce the unfolding of the host nucleoid have been isolated, characterized, and shown to map in a gene tentatively called *unf* (for "unfoldase" deficient) pending identification of the actual function of the gene product (32). The *unf* gene was subsequently shown to be the gene in which mutations occur that allow late transcription on cytosine-containing T4 phage DNA, *alc* mutations (27, 33). The evidence indicating that *alc* and *unf* mutations identify the same gene has recently been reviewed (31).

After infection of an *Escherichia coli* cell with a T4 bacteriophage, most of the metabolic processes directed by the host chromosome are rapidly and specifically inhibited (for details, see reference 31). Despite the assignment of over 75% of the coding capacity of the T4 chromosome to known genes (20, 41), the mechanisms by which phage T4 shuts off host metabolism remain unknown.

The T4 *unf* gene is of particular interest because it is the only T4 gene shown to be involved in the shutoff of host transcription by the results of *in vivo* experiments (27, 37). Two other genes, *alt* and *mod*, which control the ADP ribosylation of RNA polymerase α subunits, have been implicated in host transcription shutoff by the results of *in vitro* experiments (17; R. Drivdahl and E. Kutter, Fed. Proc. 42:1881, 1983). However, when an *alt mod* double mutant was constructed and studied *in vivo*, it was shown to shut off host rRNA synthesis with the same kinetics as wild-type T4 (9).

The *unf* gene product is known to be responsible for the shutoff of late transcription from cytosine-containing T4

DNA (27, 33) and from phage lambda DNA (23). Initially, it was thought that the *unf* gene product was also responsible for the shutoff of host transcription (27). However, subsequent experiments have indicated that, whereas shutoff of host transcription is slightly delayed in cells infected with *unf* mutants, shutoff is nevertheless essentially complete by 10 min postinfection (31, 37). It seems likely, therefore, that the *unf* gene product acts in concert with other T4 gene products to arrest host transcription. (See the recent reviews by Snustad et al. [31] and by Rabussay [24] for further details.)

Preliminary evidence (27) suggested that the *unf* gene might code for a 15,000-dalton polypeptide known to copurify with the host RNA polymerase (35). Subsequent results have not supported this possibility (31), and recent results have indicated that the *unf* gene product is a polypeptide of slightly over 18,000 daltons in size (9a, 14a). Although the results of Drivdahl and Kutter (Fed. Proc. 42:1881, 1983; personal communication) indicate that the *unf* gene product decreases the efficiency of transcription from cytosine-containing DNA templates *in vitro*, the exact function(s) of this gene product remains unknown.

The *unf* gene is unessential in the commonly used laboratory strains of *E. coli*. However, the introduction of the conjugative F1 incompatibility group plasmid pR386 Tc into these strains renders them partially to completely restrictive to the growth of *unf*⁻, but not *unf*⁺, phage (10). In the present study, comparative analyses of *unf*⁺ and *unf*⁻ phage growth in plasmid pR386-containing restrictive hosts were carried out in an attempt to further define the role of the *unf* gene product. Surprisingly, the results indicated that the *unf* gene product is required for late DNA replication when plasmid pR386 is present in the host cell and that this requirement is eliminated by the addition of chloramphenicol to infected cells after the synthesis of early phage gene products.

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MATERIALS AND METHODS

Phage and bacterial strains. The origins of *E. coli* strains B/5, CR63, and S/6/5 have been previously described (32). Plasmid pR386 was obtained from E. Lederberg, Plasmid Reference Center, Stanford University (Stanford, Calif.), in *E. coli* strain J53F⁻ (λ) *pro-22 met-63* and was transferred to *E. coli* strains S/6/5 and B/5 as previously described (10). The origins of phage mutants, preparation of stocks, construction of multiple mutants, and methods of monitoring the presence of mutations *amN82* (gene 44), *rPT8x2*, *nd28x6*, and *unf39x5* in multiple mutants have previously been described (10, 29, 32).

Media and chemicals. H-broth, EHA bottom-layer agar, and EHA top-layer agar were prepared as described by Steinberg and Edgar (34). H-broth was used to grow all bacterial overnight and indicator cultures and most phage stocks. T-broth (6) was used as a dilution medium. LB broth (18) was used to grow phage stocks for polyethylene glycol concentration. Fraser's glycerol medium (8) was used as the growth medium in [³H]thymidine and [³H]uridine labeling experiments. M-9 medium (1) was used for ³⁵S labeling experiments.

All radioactive isotopes were purchased from ICN except [¹⁴C]thymidine, which was purchased from Schwarz/Mann. Polyethylene glycol 8000 (Carbowax 6000) was purchased from Union Carbide Corp. DNase I, RNase A, protease (type XIV), and all amino acids were purchased from Sigma Chemical Co. RNase-free DNase was purchased from Worthington Diagnostics. [³H]thymidine-labeled lambda DNA was obtained from Bethesda Research Laboratories as a gift of W. Sauerbier. Ampholines were purchased from Bio-Rad Laboratories.

Preparation of phage stocks by polyethylene glycol precipitation. When labeling experiments required phage to be suspended in minimal medium or when very high titers were needed, phage grown in LB broth (18) were concentrated by the polyethylene glycol precipitation procedure of Yamamoto et al. (42) and were suspended in T4 Tris salts.

Preparation of [¹⁴C]- or [³H]thymidine-labeled phage. [¹⁴C]- or [³H]thymidine-labeled T4 phage were prepared by differential centrifugation as described by Warner et al. (40), except that the phage pellet was allowed to resuspend overnight at 4°C in T4 Tris salts (0.15 M NaCl, 0.01 M Tris, pH 7.4).

Radioactivity measurements. Radioactivity was quantitated with a Beckmann LS 7000 liquid scintillation spectrometer as previously described (37).

Isolation of DNA for hybridization. *E. coli* and T4 DNAs were prepared as described by Lee and Miller (16).

Kinetics of switchover from host transcription to phage transcription. RNA was pulse-labeled in infected *E. coli* strain S/6/5(pR386) cells as previously described (37), except that the infections were done at 27°C so that restriction of the single mutant *unf39x5* would be complete. RNA was isolated, and RNA-DNA hybridization experiments were done as previously described (37).

Breakdown of labeled parental phage DNA. *E. coli* S/6/5 and S/6/5(pR386) cultures were grown in Fraser's medium at 37°C to about 5×10^8 cells per ml (75 and 65 Klett units, respectively). The cells were collected by centrifugation ($4,340 \times g$ for 10 min at 4°C) and were suspended in one-half the original volume of cold Fraser's medium. Two-milliliter cell cultures were infected with [³H]thymidine-labeled phage (0.5-ml volume adjusted to 10^{10} phage per ml) and incubated at 30°C with aeration by shaking. At various times after

infection, 100- μ l samples were transferred to 2-cm² Whatman 3MM filter papers and quickly immersed in 1-ml volumes of ice-chilled 5% trichloroacetic acid (TCA) in glass scintillation vials. The filter paper squares were removed from the scintillation vials 10 min later, washed a second time with 5% TCA, dehydrated with ethanol, dried, and counted by scintillation spectroscopy as described earlier. TCA-soluble radioactivity was determined by adding 1-ml volumes from the first TCA washes to 10-ml volumes of scintillation cocktail containing 0.6% (wt/vol) 2,5-diphenyloxazole in a 2:1 mixture of toluene and Triton X-100.

Host DNA degradation, superinfection exclusion, and phage growth rates. The kinetics of degradation of [³H]thymidine-prelabeled host DNA, the induction of superinfection exclusion, and phage growth rates in S/6/5 and S/6/5(pR386) cells infected with *unf*⁺ and *unf*⁻ phage were measured as previously described (28, 30, 32).

Patterns of protein synthesis. *E. coli* B/5 and B/5(pR386) cells were grown in M-9 medium to a density of about 4×10^8 cells per ml (46 and 50 Klett units, respectively), collected by centrifugation for 10 min at $4,340 \times g$, washed with a 10-ml volume of M-9 medium, recentrifuged, and suspended in the original volume in M-9 medium. Cell cultures (5 ml) were prewarmed to 30°C for 2 min, infected with a multiplicity of infection of 6, and incubated with aeration by shaking at 30°C. At 2, 10, and 18 min after infection, 5-ml cultures were transferred to flasks containing 100 μ Ci of H₂³⁵SO₄ (specific activity, 43 Ci/mg) and incubated with aeration by shaking at 30°C for 6 min. Labeling was stopped by transferring the cultures to ice-chilled 15-ml Corex centrifuge tubes containing 0.1-ml volumes of 2 M (NH₄)₂SO₄. Incorporation into acid-insoluble material was determined by analyzing 20- μ l samples as described above. The pulse-labeled cells were harvested by centrifugation at $4,340 \times g$ for 10 min. They were suspended in 100- μ l volumes of electrophoresis sample buffer and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis was performed with 12% Studier-Laemmli gels (15, 36) with the buffers described by Berget and Warner (3). Samples were prepared as previously described (29) and loaded on the gels in 30- μ l volumes of electrophoresis sample buffer. In some experiments (including all of them for which data are presented in this paper), constant volumes of infected cell lysate were loaded on the gels. In other experiments, constant amounts of acid-insoluble radioactivity (30,000 cpm) were loaded. The gels were run at 20 mA (constant current) until the tracking dye had just run off the gel and were fixed in a 10% TCA-1% sulfosalicylic acid solution for a minimum of 2 h. Labeled proteins were detected by the fluorography technique of Bonner and Laskey (4), using Kodak XAR-2 X-ray film.

Two-dimensional gel electrophoresis. Two-dimensional isoelectric focusing-SDS-polyacrylamide gel electrophoresis was performed according to the procedure of O'Farrell (22) except that 20% of the pH 5 to 7 ampholine solution was replaced with pH 6 to 8 ampholine solution. To determine the pH gradient in each isoelectric focusing run, one gel was cut into 0.5-cm segments, and the pH of each segment was measured. Protein sizes were estimated by using ¹⁴C-labeled marker proteins; they were added to isoelectric focusing gel mixtures minus ampholines at a concentration yielding 30,000 cpm per 0.5-cm length of polymerized gel. When markers were used, the basic end of the focusing gel was cut off and replaced with a 0.5-cm length of marker gel, which was

bonded to the trough of the second-dimension 12% polyacrylamide gel with 1% agarose just like the focusing gel. The positions of ^{35}S -labeled proteins present in gels were recorded by the scintillation autoradiography procedure of Bonner and Laskey (4).

Kinetics of shutoff of host DNA synthesis. Cultures of *E. coli* strains S/6/5 and S/6/5(pR386) were grown to a density of about 4×10^8 cells per ml (40 Klett units) in Fraser's medium at 37°C. The cultures were allowed to equilibrate at 30°C, and 4-ml volumes were infected with 1-ml phage suspensions adjusted to yield multiplicities of infection of 5. [^3H]thymidine (25 μCi from a 1-mCi/ml solution; specific activity, 69 Ci/mmol) and uridine (25 μl of a 200-mg/ml solution) were added at the time of infection. Acid-insoluble radioactivity was measured in 50- μl samples taken at the desired intervals after infection as previously described (29).

DNA synthesis. (i) Net incorporation of [^3H]thymidine. *E. coli* S/6/5 and S/6/5(pR386) cells were grown in Fraser's medium to about 4×10^8 cells per ml. They were infected by transferring 4-ml samples to flasks containing phage suspended in T4 Tris salts (multiplicity of infection = 5), 25 μCi of [^3H]thymidine, and uridine (final concentration, 0.5 mg/ml). Labeling was done in some experiments in the presence of 5-fluorodeoxyuridine (FUDR; final concentration, 10 $\mu\text{g}/\text{ml}$) to inhibit the phage and host thymidylate synthetases. When FUDR was used, uracil (final concentra-

FIG. 1. The kinetics of shutoff of host DNA synthesis in *E. coli* strain S/6/5 (open symbols) or S/6/5(pR386) (solid symbols) after infection with the phage DNA synthesis-negative, unfoldase-proficient multiple mutant *amN82 rPT8 nd28x6* (squares) and the phage DNA synthesis-negative unfoldase-deficient multiple mutant *amN82 rPT8 nd28x6 unf39x5* (triangles) at 30°C. The kinetics of DNA synthesis in uninfected S/6/5 (○) and S/6/5(pR386) (●) are also shown.

FIG. 2. Shutoff of host RNA synthesis in *E. coli* strain S/6/5(pR386) infected with wild-type phage (T4D⁺) (○) or the unfoldase-deficient phage strain *rPT8x2 nd28x6 unf39x5* (●) at 30°C. Infected cells were pulse-labeled for 3 min with [^3H]uridine. A constant number of counts (50,000 cpm) of extracted radioactive RNA was hybridized to *E. coli* DNA samples (50 μg) that were immobilized on nitrocellulose filters.

tion, 25 $\mu\text{g}/\text{ml}$) and cold thymidine (final concentration, 10.5 $\mu\text{g}/\text{ml}$) were also added. At various times after infection, 50- μl samples were taken and analyzed for acid-insoluble radioactivity as described earlier. Total radioactivity per culture was measured by analyzing 50- μl samples taken at the conclusion of the experiment and processed as described earlier.

(ii) Pulse-labeling with [^3H]thymidine. Pulse-labeling experiments were performed as described above except that 12-ml cultures were infected and labeled for 2 min at various times after infection by transferring 1-ml samples of the cultures to scintillation vials containing 10 μCi of [^3H]thymidine. After the 2-min pulses, 50- μl samples were taken and analyzed for acid-insoluble radioactivity as described earlier. Upon completion of the experiments, 50- μl samples were analyzed for total radioactivity.

(iii) Label-chase experiments. [^3H]thymidine label-chase experiments were performed as described above except that 2-ml cell cultures were used, the cells were labeled from 6 to 18 min postinfection, and the labeling periods were terminated by adding 6×10^5 -fold excesses of cold thymidine. Samples (50 μl) were analyzed at the desired times during the labeling period and after the chase was initiated.

(iv) Effect of chloramphenicol on DNA synthesis in phage-infected S/6/5(pR386) cells. The effect of chloramphenicol on DNA synthesis in phage-infected cells was examined by using the protocol described above with 2-ml cultures except that the phage were in H-broth rather than T4 Tris salts and chloramphenicol (80 μl of a 2.5-mg/ml solution; final concentration, 100 $\mu\text{g}/\text{ml}$) was added at 11 min postinfection. Sterile distilled water (80 μl) was added in the control infections at 11 min postinfection. In addition, total radioactivity per culture was measured at 17 min postinfection rather than at the end of the experiment.

Alkaline sucrose gradient analysis of labeled phage DNA. *E. coli* S/6/5 and S/6/5(pR386) cells were grown in Fraser's medium to 4×10^8 cells per ml (40 Klett units). Cell cultures (5 ml) were infected (multiplicity of infection = 5) and labeled (50 μCi of [^3H]thymidine) in the presence of uridine

FIG. 3. Kinetics of degradation of prelabeled host DNA. *E. coli* strain S/6/5 (open symbols) or S/6/5(pR386) (solid symbols) cells were infected with the phage DNA synthesis-negative mutant *amN82* (circles) or the phage DNA synthesis-negative, *Unf*⁻ multiple mutant *amN82 rPT8x2 nd28x6 unf39* (triangles) at 30°C.

(final concentration, 0.5 mg/ml). At various times after infection, 1-ml samples were diluted into 3-ml volumes of ice-chilled TNE (0.01 M Tris, 0.015 M Na₂ EDTA, 0.15 M NaCl, pH 7.4). Percent incorporation was determined by analyzing 50- μ l samples for acid-insoluble and total radioactivity.

Cells in 0.2-ml samples in TNE were lysed by adding freshly prepared lysozyme (final concentration, 20 μ g/ml), placing them on ice for 20 min, adding Sarkosyl (10% solution) to a final concentration of 1%, and placing them on ice for another 20 min. NaOH was then added (final concen-

tration, 0.2 M), and the lysates were incubated for 20 min at room temperature. Samples (0.2 ml) were layered onto 5 to 20% alkaline sucrose gradients prepared as described by Jacob and Hobbs (13) and overlaid with paraffin oil. The gradients were centrifuged with a Beckman SW50.1 rotor in a Beckman L5-50 or L5-65 centrifuge for 75 min at 40,000 rpm (10°C). The speed was increased slowly up to 5,000 rpm before being accelerated to full speed and was decreased at the end of the run without braking. Each gradient was analyzed by collecting 30 6-drop fractions from the bottom of the tube onto 2-cm² Whatman 3MM filter papers impaled on stainless-steel pins. Smooth displacement of the gradients from the centrifuge tubes was effected by pumping paraffin oil onto the top of the gradient with a Sage pump equipped with a 50-ml syringe. The filters were processed and acid-insoluble radioactivity was measured as described earlier. Each centrifugation run contained one gradient overlaid with [¹⁴C]thymidine-labeled mature T4 phage DNA or [³H]thymidine-labeled phage lambda DNA as a molecular weight marker.

RESULTS

We have attempted to examine as many parameters of phage growth as possible in an attempt to determine the earliest effect of the *unf39x5* mutation on growth in host cells containing plasmid pR386. Adsorption was previously shown not to be affected (10).

Injection and early gene expression. Penetration of parental phage DNA and early gene expression were examined by measuring the degradation of labeled parental phage DNA to acid-soluble products, the onset of superinfection exclusion, shutoff of host DNA and RNA syntheses, and degradation of prelabeled host DNA.

No differences were detected in the amounts of labeled *unf*⁺ and *unf*⁻ parental phage DNA converted to acid-soluble material or in the rates of onset of superinfection exclusion after infection of *E. coli* strains S/6/5 and S/6/5(pR386) (data not shown). Moreover, host DNA synthesis was shut off as rapidly and completely by *unf*⁺ or *unf*⁻ phage whether the host was strain S/6/5 or S/6/5(pR386) (Fig. 1). Surprisingly, host RNA synthesis seemed to be shut off

FIG. 4. Growth rates of T4D⁺ (○), the unfoldase-proficient double mutant *rPT8x2 nd28x6* (△), the unfoldase-deficient single mutant *unf39x5* (◇), and the unfoldase-deficient multiple mutant *rPT8x2 nd28x6 unf39x5* (□) in *E. coli* strain S/6/5 (A) or *E. coli* strain S/6/5(pR386) (B) at 30°C.

as rapidly in *unf39x5*-infected as in wild-type T4-infected S/6/5(pR386) cells (Fig. 2). Since our main concern in this study was to exclude the possibility that *unf*⁻ phage might not be able to shut off host transcription when plasmid pR386 was present, we did not attempt to examine the kinetics of shutoff more carefully to see whether there was really a brief delay in shutoff as documented earlier (37) in plasmid-free host cells. Finally, the rates of degradation of prelabeled S/6/5(pR386) DNA were the same whether the infecting phage was *unf*⁺ or *unf*⁻ (Fig. 3). These results all indicate that *unf*⁻ chromosomes are injected and initiate early gene expression just as efficiently as *unf*⁺ chromosomes in strain S/6/5(pR386).

Growth rates. We next examined the growth rates in terms of maturation (appearance of intracellular progeny phage) of *unf*⁺ and *unf*⁻ phage in the *unf*-permissive strain S/6/5 and the *unf*-restrictive strain S/6/5(pR386). The results (Fig. 4) showed no difference in the growth rates of *unf*⁺ and *unf*⁻ phage in S/6/5, but did show inhibition of growth of *unf*⁻ phage in S/6/5(pR386). These experiments were carried out at 30°C, a temperature at which restriction of the unfoldase-deficient, nuclear disruption-deficient, endonuclease II and IV-deficient strain *rPT8x2 nd28x6 unf39x5* is complete, but restriction of the *unf39x5* single mutant is only partial (10). Tighter restriction of the *unf39x5* single mutant is observed at 27°C (10). When grown in S/6/5(pR386) at 30°C, the average burst size was 7 for *unf39x5* and <1 for *rPT8x2 nd28x6 unf39x5*. Maturation commenced at about the same time and rate in *unf*⁻ infections as in *unf*⁺ infections; however, in *unf*⁻ infections, it slowed quickly and was essentially complete by 40 min postinfection (Fig. 4). Single-burst experiments were also done, and they showed that the low average burst size in *unf*⁻ infections resulted from the production of only a small number of progeny phage per yielder (infected cell producing at least one progeny phage)

FIG. 5. Kinetics of phage-induced RNA synthesis in *E. coli* strain S/6/5(pR386) infected with T4D⁺ (○) or the unfoldase-deficient multiple mutant *rPT8x2 nd28x6 unf39x5* (□). Infected cells were pulse-labeled for 3 min with [³H]uridine, the nucleic acids were extracted, DNA was removed with DNase (RNase-free), and a constant amount of radioactive RNA (50,000 cpm) was hybridized to T4D⁺ DNA samples (25 µg) immobilized on nitrocellulose filters.

FIG. 6. SDS-polyacrylamide gel electrophoretic patterns of ³⁵S-labeled proteins in whole-cell extracts of *E. coli* strain B/5 (A) or B/5(pR386) (B), in both cases either uninfected or infected with the unfoldase-proficient double mutant *rPT8x2 nd28x6* or the unfoldase-deficient triple mutant *rPT8x2 nd28x6 unf39x5*. Constant volumes of infected cell lysate were loaded on the gel shown.

rather than a few large bursts in conjunction with a decreased proportion of yielders (data not shown).

RNA synthesis. Hybridization of pulse-labeled RNA extracted from *unf*⁺ and *unf*⁻ phage-infected S/6/5(pR386) cells to phage T4 DNA showed that comparable amounts of phage RNA were synthesized in both cases (Fig. 5). The slightly reduced level of phage RNA synthesized in *unf*⁻ phage-infected cells labeled from 3 to 6 min after infection is probably the result of the previously documented slower switchover from host RNA synthesis to phage RNA synthesis in *unf*⁻ infections (37). The apparent decrease in T4-specific RNA synthesis during the 9- to 12-min and 12- to 15-min labeling periods is probably not real. Rather, the observed decrease in amounts of labeled RNA in T4 DNA-RNA duplexes is almost certainly due to competition with increased quantities of unlabeled RNA synthesized before the pulse. Much higher DNA/RNA ratios would have to be used to eliminate the effect of competing unlabeled endogenous RNA, the concentration of which increases with increasing time post-infection.

Patterns of protein synthesis. The patterns of ³⁵S-labeled phage proteins synthesized in S/6/5 or B/5 and S/6/5(pR386) or B/5(pR386) cells infected with the *Unf*⁺ phage *rPT8x2 nd28x6* and the *Unf*⁻ phage *rPT8x2 nd28x6 unf39x5* were examined by comparing autoradiograms of SDS-polyacrylamide gels of proteins in extracts of cells labeled at

FIG. 7. Two-dimensional isoelectric focusing-SDS-polyacrylamide gel electrophoresis patterns of ^{35}S -labeled proteins synthesized from 5 to 25 min after infection of *E. coli* strain B/5(pR386) at 30°C with rPT8x2 nd28x6 (A) or rPT8x2 nd28x6 unf39x5 (B). The positions of ^{14}C -labeled α -chymotrypsinogen (molecular weight, 25,700) and β -lactoglobulin (molecular weight, 18,400) are shown on the left. The position of the *unf* protein is shown in (A).

various times after infection. Representative patterns observed for B/5 and B/5(pR386) cells labeled from 2 to 8, 10 to 16, and 18 to 24 min postinfection are shown in Fig. 6. We were particularly interested in whether the switch from early protein synthesis to late protein synthesis occurred normally, since the *unf* gene product has been implicated in transcriptional control, and in whether all of the essential T4 proteins were synthesized. The results clearly showed that early protein synthesis was turned off and late protein

synthesis was turned on with approximately the same kinetics in the absence of plasmid pR386 regardless of whether the infecting phage was *unf*⁺ or *unf*⁻ (Fig. 6A). The switch-over from early to late protein synthesis also occurred with similar kinetics in plasmid-containing cells infected with either *unf*⁺ or *unf*⁻ phage, although it appeared to be somewhat slower in *unf*⁻ phage-infected cells (Fig. 6B). In any case, the switchover was clearly much slower in plasmid-containing cells than in plasmid-free cells regardless of the genotype of the infecting phage (cf. Fig. 6A and B).

The different kinetics of switchover from early to late protein synthesis in plasmid-containing and plasmid-free cells make quantitative comparisons of the results of pulse-labeling experiments very difficult. A protein will appear to be synthesized in lower amounts if its synthesis begins later in the pulse period. Since a considerable proportion of the T4 immediate-early proteins are small proteins and many of the largest T4 proteins are late proteins, the slow switchover from early to late protein synthesis could cause the results of pulse-labeling experiments to appear to show the overproduction of small proteins and the underproduction of large proteins even if this is not the case. Thus, two-dimensional isoelectric focusing-SDS-polyacrylamide gel electrophoresis was used to compare the proteins synthesized in *unf*⁺ and *unf*⁻ phage-infected B/5(pR386) cells labeled continuously from 5 to 25 min postinfection. The results (Fig. 7) indicated that very similar patterns of protein synthesis occurred in plasmid-containing cells infected with *unf*⁺ and *unf*⁻ phage. The absence of any essential protein or class of proteins was not evident in B/5(pR386) cells infected with *unf*⁻ phage.

DNA synthesis. Phage DNA synthesis was first examined by measuring the net incorporation of [^3H]thymidine into acid-insoluble material in S/6/5 and S/6/5(pR386) cells infected with *unf*⁺ and *unf*⁻ phage. The results showed that net incorporation of [^3H]thymidine essentially stops at 20 to

FIG. 8. Rates of DNA synthesis; net incorporation of [^3H]thymidine. Acid-insoluble radioactivity was measured from uninfected (●) *E. coli* strain S/6/5 (A) or S/6/5(pR386) (B), and from S/6/5 (A) or S/6/5(pR386) (B) infected with T4D⁺ (○), the unfoldase-deficient double mutant rPT8x2 nd28x6 (△), the unfoldase-deficient single mutant unf39x5 (◇), or the unfoldase-deficient multiple mutant rPT8x2 nd28x6 unf39x5 (□) at 30°C.

FIG. 9. Rates of DNA synthesis; pulse-labeling with [3 H]thymidine in the presence of FUDR. Acid-insoluble radioactivity was measured after 2-min pulse-labeling periods for uninfected *E. coli* (Δ) strain S/6/5 (A) or S/6/5(pR386) (B) and from S/6/5 (A) or S/6/5(pR386) (B) infected with T4D $^+$ ($\circ$) or the unfoldase-deficient single mutant *unf39x5* ($\diamond$) at 30°C.

30 min after infection of S/6/5(pR386) cells at 30°C with the completely growth-inhibited phage *rPT8x2 nd28x6 unf39x5* (Fig. 8B). The rate of DNA synthesis is also slow in S/6/5(pR386) cells infected with the *unf* $^+$ double mutant *rPT8x2 nd28x6*; however, in this case, DNA synthesis does continue throughout the latent period and large numbers of progeny phage are produced (Fig. 4B).

Although the single mutant *unf39x5* is only partially inhibited at 30°C, *unf39x5*-infected S/6/5(pR386) cells also showed a sharp decline in net incorporation of [3 H]thymidine at 20 to 30 min postinfection (Fig. 8B). This *unf*-specific decline in incorporation was observed only in the presence of plasmid pR386; it did not occur in *unf39x5*-infected S/6/5 cells (Fig. 8A). When this same experiment was repeated in the presence of the thymidylate synthetase inhibitor FUDR, the same plasmid-specific inhibition of net incorporation of [3 H]thymidine was observed (data not shown). Thus, the effect was not the result of differential expression of the T4 *td* (thymidylate synthetase) gene of the different phage strains in S/6/5(pR386) cells.

The rates of DNA synthesis occurring at various times after infection of S/6/5 and S/6/5(pR386) cells with *unf* $^+$ and *unf* $^-$ phage were subsequently examined by pulse-labeling experiments. The amounts of [3 H]thymidine incorporated into acid-insoluble material during 2-min pulses in the presence of FUDR were measured. The results (Fig. 9) indicated that the arrest of net incorporation observed in S/6/5(pR386) cells infected with *unf* $^-$ phage was due to the termination of DNA synthesis rather than an increased rate of degradation of newly synthesized DNA. This was further verified by the

results of label-chase experiments (Fig. 10). Lastly, the addition of chloramphenicol to *unf* $^-$ phage-infected S/6/5(pR386) cells at 11 min postinfection of 30°C has been shown to prevent the premature termination of phage DNA synthesis in these cells (Fig. 11).

Alkaline sucrose gradient analysis of intracellular phage DNA. The intracellular pools of T4 DNA were examined by alkaline sucrose gradient centrifugation to determine whether the replicative DNA might become nicked or cleaved in S/6/5(pR386) cells infected with *unf* $^-$ phage. The results (Fig. 12) showed no evidence of nicking or cleavage of *unf* $^+$ or *unf* $^-$ DNA in either S/6/5 or S/6/5(pR386). The only apparent difference was the absence of significant amounts of mature phage size DNA molecules at late times after infection of S/6/5(pR386) with *unf* $^-$ phage. There was no detectable accumulation of DNA single strands that were reduced in length in S/6/5(pR386) cells infected with *unf* $^-$ phage compared with *unf39x5*-infected S/6/5 cells or wild-type T4-infected S/6/5(pR386) or S/6/5 cells.

DISCUSSION

In the presence of plasmid pR386, the growth of *Unf* $^-$ phage was inhibited, even though the phage appeared to adsorb, inject their DNA, and express early gene functions as efficiently as *Unf* $^+$ phage. This description fits the definition of plasmid-induced abortive infection (5). Mutants of phage T4 have been shown in the past to abortively infect certain strains of *E. coli*. The classic example of this is the abortive infection of phage lambda lysogens by *rII* mutants

FIG. 10. Rates of DNA synthesis; [3 H]thymidine label-chase experiments with uninfected *E. coli* ($\circ$) strain S/6/5 (A) or S/6/5(pR386) (B) and S/6/5 (A) or S/6/5(pR386) (B) infected with the unfoldase-proficient double mutant *rPT8x2 nd28x6* (Δ) or the unfoldase-deficient multiple mutant *rPT8x2 nd28x6 unf39x5* ($\square$) at 30°C. The radiolabel was added at 6 min after infection, and at 18 min after infection the label was chased with excess cold thymidine.

FIG. 11. Effect of chloramphenicol on DNA synthesis. The effect of the addition of chloramphenicol at 11 min postinfection at 30°C on DNA synthesis is shown for *E. coli* strain S/6/5(pR386) cells infected with T4D⁺ (A) and *unf*39x5 (B). Symbols: without chloramphenicol (□); with chloramphenicol (○).

(2). Even wild-type T4 has been found to abortively infect *E. coli* strains containing plasmid pRts1 at 32°C (12). The unique aspect of the *unf*⁻ phage-plasmid pR386 interaction discussed in this paper is that an otherwise nonessential T4 gene becomes essential in the presence of a particular plasmid.

In the present study, we have attempted to use plasmid pR386-containing *unf*-restrictive hosts to gain additional information about the function of the *unf* gene. Prior studies have implicated the *unf* gene in the unfolding of the host nucleoid (27, 32), the inhibition of late transcription from C-containing T4 DNA (33) and lambda DNA (23), and the rapid shutoff of host transcription (27, 37). We thus expected to observe some type of defect in the regulation of transcription in *unf*39x5-infected cells containing plasmid pR386, for example, the inability to turn on late transcription. Our results showed that early functions are turned off and late functions are turned on slowly in plasmid pR386-containing cells regardless of whether the infecting phage is Unf⁺ or Unf⁻. The switchover appeared to be slightly slower in *unf*⁻ phage-infected cells, and it is conceivable that this slow switchover is the basis of the plasmid-directed restriction of *unf*⁻ phage growth. However, the results of two-dimensional polyacrylamide gel electrophoresis showed that very similar patterns of protein synthesis occur over the course of the entire latent period in *unf*⁺ and *unf*⁻ phage-in-

fecting B/5(pR386) cells. Clearly, the results showed that the switch from early protein synthesis to late protein synthesis is operational, although possibly slow, in these *unf*39x5-infected cells.

On the other hand, phage DNA synthesis began normally, but then stopped at 20 to 30 min after infection of plasmid pR386-containing cells with *unf*⁻ phage. Pulse-chase and alkaline sucrose gradient experiments gave no indication of abnormal nicking, cleavage, or degradation of the nascent DNA. Thus, it seems very unlikely that plasmid pR386 encodes a nuclease active only on *unf*⁻ phage DNA or synthesized only in *unf*39x5-infected cells.

Considerable evidence (see recent reviews by Kozinski [14] and Mosig [19]) indicates that T4 DNA replication is initiated de novo at specific origins and at recombination branch points. Primary initiation probably occurs at specific origin sequences, whereas late replication appears to be initiated from recombination intermediates (21). If this is the case, our results could be explained if the *unf* gene product is required for initiation of late replication from recombination junctions when plasmid pR386 is present in the host cell. In fact, the kinetics of the arrest of DNA synthesis in *unf*⁻ phage-infected S/6/5(pR386) cells are very similar to those observed in restrictive host cells infected with "DNA arrest" amber mutants, such as those with mutations in genes 46 and 47 (7, 39). In the case of these mutants, the premature termination of DNA synthesis can be prevented by the addition of chloramphenicol to the infected cells after permitting the synthesis of the essential DNA replicative proteins or by the addition of a mutation in gene 55 which prevents the turn off of the early unique-origin mode of replication (11, 25, 26). Our results have shown that the addition of chloramphenicol to *unf*⁻ phage-infected S/6/5(pR386) cells at 11 min postinfection at 30°C completely eliminates the premature termination of phage DNA synthesis. This supports the possibility that the *unf* protein may be required for replication from recombination junctions when plasmid pR386 is present in the host cell. However, the situation does not appear to be this simple. Our preliminary data indicate that the premature arrest of DNA synthesis does occur in S/6/5(pR386) cells infected with an *amBL*292 (gene 55)-*unf*39x5 double mutant. Clearly, additional studies are needed to determine the basis of the aborted DNA synthesis in *unf*39x5-infected plasmid-containing cells. In particular, the rifampin sensitivity of the observed DNA synthesis needs to be examined in *unf*⁻ phage-infected plasmid-containing cells.

Pearson and Snyder (23) were unable to detect *unf* gene product topoisomerase activity when supercoiled phage lambda DNA was used as substrate. This, of course, does not exclude the possibility that the *unf* protein might be active as a topoisomerase on the folded genome of the host or on a replicative form of T4 DNA.

We attempted to purify plasmid pR386 DNA by several different procedures to determine whether T4 *unf*⁺ and *unf*⁻ phage shut off replication and transcription of the plasmid in infected cells. Regardless of the purification procedure used, cross-hybridization between plasmid pR386 and chromosomal DNA was always observed. We subsequently showed that the introduction of plasmid pR386 into *met*⁻ host cells converted them to *Met*⁺. Thus, pR386 is probably an F' carrying host chromosomal DNA sequences. In any case, we were not able to determine the effect of T4 infection on the replication or the transcription of the plasmid itself. This would have to be done with clones of plasmid sequences not cross-hybridizing with chromosomal sequences or with

FIG. 12. Alkaline sucrose gradient analysis of intracellular phage DNA. The gradients shown are for infected cells lysed at 20 min after infection (A), 40 min after infection (B), 40 min after infection of cells superinfected at 8 min (C), and 90 min after infection of cells superinfected at 8 min (D). Symbols: S/6/5 infected by T4D⁺ (---); S/6/5 infected by the unfoldase-deficient single mutant *unf39x5* (···); S/6/5(pR386) infected by T4D⁺ (—); S/6/5(pR386) infected by *unf39x5* (- · - ·); S/6/5(pR386) infected by an unfoldase-proficient revertant of *rPT8x2 nd28x6 unf39x5* (—); T4 DNA marker, (↓); lambda DNA marker, (↓).

plasmids from which the chromosomal sequences had been deleted.

The results reported in this paper showed that phage DNA synthesis aborts between 20 and 30 min postinfection at 30°C in plasmid pR386-containing cells in the absence of a functional *unflalc* gene product. This premature termination of phage DNA synthesis is prevented by the addition of chloramphenicol at 11 min postinfection at 30°C. Whether the *unflalc* gene product is directly involved in late DNA synthesis or whether its function is prerequisite to the expression of another gene or set of genes required for late replication in the presence of the plasmid remains unknown. The possibility that certain essential proteins are not synthesized in *unf*⁻ phage-infected cells in the presence of plasmid pR386 cannot be ruled out even though the patterns of proteins synthesized in these cells appeared normal when examined by two-dimensional polyacrylamide gel electrophoresis.

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