JOURNAL OF VIROLOGY, Dec. 1977, p. 775-785

Vol. 24, No. 3
Printed in U.S.A.

Slow Switchover from Host RNA Synthesis to Bacteriophage RNA Synthesis After Infection of *Escherichia coli* with a T4 Mutant Defective in the Bacteriophage T4-Induced Unfolding of the Host Nucleoid

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Received for publication 22 July 1977

Most, if not all, host RNA synthesis was shut off after infection of *Escherichia coli* strain B/5 with a bacteriophage T4 multiple mutant defective in the abilities to induce (i) unfolding of the host nucleoid (*unf*⁻), (ii) nuclear disruption (*ndd*⁻), and (iii) host DNA degradation (*den*A⁻, *den*B⁻). The shutoff of host RNA synthesis and turn-on of phage RNA synthesis were slower after infection of *E. coli* with *unf*⁻ phage than after infection with *unf*⁺ phage. This delay in the switchover from host RNA synthesis to phage RNA synthesis in *unf*⁻ infections did not result in a measurable delay in the onset of nuclear disruption, deoxyribonucleoside monophosphate kinase synthesis, or DNA synthesis. *unf*39 did not complement *alc* (*a*llows *l*ate transcription on *c*ytosine-containing DNA) mutants, supporting the proposal of Sirotkin et al. [Nature (London) **265**:28-32, 1977] that *alc* and *unf* are possibly the same gene.

At the time of infection of an *Escherichia coli* cell with a T4 bacteriophage, that cell is presented with two sets of genetic instructions dictating two completely different pathways of metabolic activity. At least in the case of the commonly studied *E. coli* strains grown under standard laboratory conditions, such T4-infected cells respond invariably to the dictations of the viral genome. What is the molecular basis of the absolute genetic control of such a virulent pathogen over the host's metabolic machinery? At present, very little is known about the genetics of such host-parasite interrelationships.

In an attempt to elucidate the basis of the genetic control of the *E. coli* metabolic machinery by the infecting T4 genophore, we and others have isolated and studied T4 mutants deficient in each of the known T4-induced modifications of the host genome (10, 33, 35, 46). The known modifications include: (i) "unfolding" of the host chromosomes (loss of the RNA-maintained, negatively supercoiled loops, or "domains") (34, 35, 41, 43, 51), (ii) "nuclear disruption" (movement of the host DNA into juxtaposition with the cell membrane) (2, 12, 22, 24, 33, 36), and (iii) degradation of the majority of the host DNA to mononucleotides and their subsequent reincorporation into phage DNA (11, 16-18, 48).

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Host DNA, RNA, and protein syntheses are shut off normally after infection of *E. coli* with T4 mutants that fail to induce degradation of host DNA (31, 32; H. R. Warner, personal communication). Host RNA and protein syntheses are also shut off normally after infection of *E. coli* with nuclear disruption-deficient mutants (31, 32). The shutoff of host DNA synthesis, however, is delayed until about 10 min after infection of *E. coli* with nuclear disruption-deficient T4 phage (32). We have recently shown that a mutant (*unf*39) that is defective in the T4-induced unfolding of the folded nucleoids of *E. coli* (41, 51) induces normal shutoff of host DNA and protein synthesis (35).

The isolated folded genomes of *E. coli* have approximately the same dimensions as the cell nucleoids in vivo and are excellent templates for RNA synthesis (28). Moreover, treatment of cells with rifampin results in unfolding of the chromosomes (27), suggesting that ongoing transcription is necessary to maintain the folded structures. It thus seemed likely that a T4 phage mutant that failed to induce the unfolding of host nucleoids (35) might do so because of its failure to inhibit host RNA synthesis. We have examined this possibility directly by DNA-RNA hybridization studies of RNA pulse-labeled at various times after infection of *E. coli* cells with *unf*⁺ and *unf*⁻ phage.

In this paper, we report results that show that

most host RNA synthesis is shut off in the absence of unfolding of the host nucleoid, nuclear disruption, and host DNA degradation. The results indicate, however, that the shutoff of host RNA synthesis and the turn-on of phage RNA synthesis are slower when the infecting genophore carries an "unfoldase-defective" (*unf*) mutation. The slow turn-on of phage RNA synthesis does not result in a measurable delay in nuclear disruption, deoxyribonucleoside monophosphate kinase synthesis, or DNA synthesis, although we earlier showed that it does result in a corresponding delay in the degradation of host DNA and in progeny maturation (35).

Results are reported that support the proposal of Sirotkin et al. (29) that *unf* and *alc* are the same gene. DNA-RNA hybridization data are presented that are consistent with the conclusion of Sirotkin et al. (29) that the *alc/unf* mutants are defective in the shutoff of synthesis of at least some species of *E. coli* RNA. Our results are not consistent, however, with their estimates of the relative amounts of *E. coli*- and T4-specific RNA synthesis occurring after infection with *alc/unf* mutants.

MATERIALS AND METHODS

Phage and bacterial strains. The T4 mutant strains *am*E51-DD2-*alc*1 and *am*E51-DD2-*alc*3 (29, 37) were provided by L. Snyder. T4 mutant *r*NB2226 was obtained from C. G. Goff. The origins of all *E. coli* and all other phage strains (except the spontaneous *unf* mutants), the construction of multiple-mutant strains, and the procedures used to grow and verify the genotypes of the phage strains used in this study have been described earlier (32, 33, 35).

Media and chemicals. H-broth, EHA bottom-layer agar, and EHA top-layer agar were prepared as described by Steinberg and Edgar (38). H-broth was used to grow all bacterial overnight and indicator cultures and phage stocks. T-broth (6) was used as a dilution medium. M63 medium (26) supplemented with Casamino Acids (5 g/liter) or Fraser glycerol medium (7) was used as the growth medium in [^{3}H]-thymidine or [^{3}H]uridine labeling experiments.

Bovine pancreatic DNase I, bovine pancreatic RNase A, bovine pancreatic RNase-free DNase I, and lysozyme were purchased from Worthington Biochemicals Corp. [^{3}H]thymidine, [^{3}H]uridine, and [^{14}C]-dTMP were purchased from Schwarz/Mann.

Isolation of spontaneous *unf* mutants. We have isolated numerous spontaneous *unf* mutants by a procedure based on the results of Snyder and colleagues (29, 37). This procedure selects for mutants of phage T4 that are capable of late transcription from cytosine-containing DNA and, thus, growth in *am su*$^-$ hosts despite the presence of a gene 56 (dCTPase-dUTPase [45, 49]) amber mutant. Many of these mutants, termed *alc* mutants (37), are *unf*$^-$ (29; see Results). We have isolated *alc* mutants in a nuclear disruption-deficient, host DNA degradation-deficient (*r*PT8x2-*nd*28x6) genetic background so that we can score unfolding of the host nucleoids in infected cells unambiguously by the pour test (43). Pinpoint plaques obtained by plating *am*E56-*r*PT8x2-*nd*28x6 phage on *E. coli* S/6/5 indicator and incubating at 27°C were punched out and replated on S/6/5 indicator at 27 and 43°C. Clones incapable of yielding visible plaques at 43°C (29, 37) were selected and plated on a lawn of the dUTPase-deficient, *N*-glycosidase-deficient *E. coli* strain BW212 (*dut*$^-$, *ung*$^-$) (44; B. Duncan and H. R. Warner, manuscript in preparation). A plaque formed on BW212 was punched out, suspended in T-broth, diluted, and replated on BW212 (*N*-glycosidase-deficient) and on *E. coli* strain CR63 (*N*-glycosidase-positive). High-titer clonal stocks of those isolates that could not grow on CR63 after passage through BW212 were prepared and scored for *unf* by the pour test as previously described (35). Revertants of *am*E56 are capable of growth on *N*-glycosidase-positive hosts after passage through dUTPase-deficient hosts because the T4 dCTPase-dUTPase (gene 56 product) prevents incorporation of uracil into progeny DNA. *alc* mutants, on the other hand, are unable to grow on *N*-glycosidase-positive hosts after passage through dUTPase-deficient hosts due to the incorporation of uracil into progeny DNA in the dUTPase-deficient host and the very rapid degradation of this uracil-containing DNA after its injection into *N*-glycosidase-positive hosts (Duncan and Warner, manuscript in preparation).

Isolation of T4 and *E. coli* DNA for hybridization experiments. *E. coli* DNA was purified from 1 liter of strain B/5 cells grown to early stationary phase at 37°C in M63 medium with Casamino Acids. The cells were pelleted by centrifugation and suspended in a final volume of 28.2 ml of TNEA buffer (0.01 M Tris-chloride [pH 7.4]–0.15 M NaCl–15 mM EDTA–1 mM NaN_3). Aliquots were transferred to four 30-ml Corex centrifuge tubes, and the cells in each tube were lysed in the presence of bovine pancreatic RNase A by adding 0.2 ml of lysozyme solution (14.4 mg/ml–50 mM EDTA–0.12 M Tris-chloride [pH 8]) and 0.8 ml of an aqueous solution of RNase A (400 μg/ml, boiled 15 min) and freeze-thawing three times in a solid CO_2-acetone bath. Sodium dodecyl sulfate was added to a final concentration of 2%, and the lysates were incubated for 1 h at 37°C. NaCl was then added (final concentration, 2 M), and the lysates were extracted on a rotating tube rack with frequent stirring with a spatula with an equal volume of water-saturated, redistilled phenol. After 30 min of phenol extraction, a volume of chloroform-isoamyl alcohol (24:1) equal to that of the lysate was added, and the extraction was continued for another 30 min. The extraction mixture was then centrifuged at 10,000 rpm for 10 min in a Sorvall HB-4 swinging-bucket rotor, and the aqueous phase was transferred with a sawed-off Pasteur pipette to a small beaker. The DNA was then precipitated by adding 2 volumes of cold 95% ethanol and wound out on a glass stirring rod. After the ethanol had evaporated, the DNA was dissolved in 15 ml of water containing a few drops of 0.25 M EDTA. The DNA solution was then subjected to a second treatment with pancreatic RNase A (final concentration, 40 μg/ml; 1 h; room temperature), a second phenol–chloroform-isoamyl alcohol extraction, and a

third extraction with just chloroform-isoamyl alcohol. The DNA was then precipitated from the aqueous phase a second time with cold 95% ethanol, wound out, and redissolved as before. The DNA solution was then dialyzed twice for 12 h each against 3 liters of 6× SSC (1× SSC = 0.15 M NaCl + 0.015 M sodium citrate), three times for 24 h each against 2 liters of 1× SSC, and once for 6 h against 3 liters of 0.1× SSC. The dialyzed DNA solution was stored frozen until used.

T4 phage DNA was similarly extracted from phage prepared by differential centrifugation from a high-titer lysate previously treated with bovine pancreatic DNase I for 20 min at 37°C.

In some of the experiments, DNA was prepared by the sodium dodecyl sulfate-Pronase-phenol-ether procedure described in detail by Lee and Miller (20).

The concentration of DNA in a preparation was determined by the absorbance of a diluted sample at 260 nm, using an extinction coefficient of 6,740 liters/mol per cm, and by the diphenylamine assay (3).

Pulse labeling and isolation of RNA. An *E. coli* B/5 culture was grown to about 4×10^8 cells per ml in M63 medium with Casamino Acids or Fraser glycerol medium at 37°C in a gyratory shaker bath. The cells were pelleted by centrifugation and adjusted to 4.4×10^8 cells per ml in fresh medium on the basis of Petroff-Hausser cell counts. Nine-milliliter subcultures were transferred to 125-ml fluted Erlenmeyer flasks containing 200 μg of tryptophan and incubated at 30°C with aeration by shaking. Thirty seconds later, 1 ml of appropriate phage suspension (final multiplicity of infection, 6) was added to each flask, excluding the uninfected control. At various times thereafter, 250 μl of [^{3}H]uridine (1 mCi/ml; specific activity, 4 Ci/mmol) was added. The pulses (1 min in one experiment, 2 min in the other experiments) were terminated by transferring 9.5-ml samples into ice-chilled centrifuge tubes containing equal volumes of TNEA buffer. After mixing the samples thoroughly, the cells were pelleted by centrifugation, suspended in 1-ml volumes of 0.01 M Tris-chloride (pH 7.2)–1 mM $MgCl_2$–1 mM NaN_3 buffer, and lysed by freeze-thawing three times in a solid CO_2-acetone bath in the presence of pancreatic RNase-free DNase I (final concentration, 40 μg/ml). Sodium dodecyl sulfate was added to a final concentration of 0.5%, and the lysates were incubated at 37°C for 10 min. Pronase (0.2 ml of a 3.2-mg/ml solution in TNEA buffer, predigested for 1 h at 37°C) was then added, and the incubation was continued at 37°C for 5 h. Nucleic acids were extracted from the lysates as described for the isolation of DNA earlier except that the 2 M NaCl step was omitted. In addition, after the first extraction, the nucleic acids were precipitated by adding 2 volumes of ethanol after the addition of 0.5 volume of 20% sodium acetate solution (pH 5.2), storing the mixture overnight at −20°C, and centrifuging it for 10 min at 10,000 rpm in a Sorvall SS34 head. The precipitates were then dissolved in 1 ml of water containing 1 drop of 0.25 M EDTA. DNA was removed by treatment with pancreatic RNase-free DNase I (final concentration, 40 μg/ml) for 30 min at 37°C followed by reextraction, once with phenol-chloroform-isoamyl alcohol and once with just chloroform-isoamyl alcohol, precipitation with ethanol and sodium acetate, and dialysis of the redissolved precipitate. The RNA samples were then stored at 4°C until used.

The concentration of RNA was determined by the orcinol reaction (5).

DNA-RNA hybridization. Hybridization of labeled RNA to *E. coli* and T4 DNA was done on cellulose nitrate filters (Millipore Corp.; HAWP, 0.45-μm pore size) as described by Denhardt (4), except that denaturation was by alkali as described by Kennell and Kotoulas (15) and in some experiments the volume of preincubation mixture added was 0.5 rather than 3.0 ml. Each hybridization vial contained a filter charged with 100 μg of *E. coli* DNA, a filter charged with 50 μg of T4 DNA, and a blank but similarly processed filter. In the five critical experiments comparing *unf*$^+$ and *unf*$^-$, each hybridization vial received 0.1 μg of RNA (25 μl of 4 μg/ml in 6× SSC) yielding DNA/RNA ratios of 10^3 and 5×10^2 for *E. coli* DNA and T4 DNA, respectively. In the survey experiments with various *alc/unf* mutants, the amount of RNA added was increased (0.4 μg in one experiment, 1 μg in another experiment; see footnotes to tables). Identical 25-μl samples were precipitated on Whatman GF/A glass-fiber disks with cold 5% trichloroacetic acid to determine the total radioactivity added to each hybridization vial.

In two experiments, pancreatic RNase A (20 μg/ml, 1 h, room temperature) was used to hydrolyze nonspecifically bound RNA after hybridization but before washing the filters (8). RNase treatment was omitted in the other experiments. There was no statistically significant difference in the amount of label bound nonspecifically between experiments where RNase was used and experiments where it was omitted. After washing the filters under low suction as described by Denhardt (4), they were dried for 2 h at 80°C and counted in 10 ml of toluene–0.6% 2,5-diphenyloxazole scintillation fluid with a Beckman LS-100C liquid scintillation spectrometer.

Kinetics of nuclear disruption. The time of occurrence of nuclear disruption in populations of *unf*$^+$ phage- and *unf*$^-$ phage-infected cells was determined by fixing cells at various times after infection and scoring the frequency of cells showing nuclear disruption in several fields of cells spread on gelatin and examined by phase microscopy as previously described (33).

Induction of deoxyribonucleoside monophosphate kinase. The kinetics of induction of T4 deoxyribonucleoside monophosphate kinase was determined by measuring the conversion of [^{14}C]dTMP (specific activity, 0.25 Ci/mol) to [^{14}C]dTDP and [^{14}C]dTTP, using extracts prepared at various times after infection of *E. coli* B/5 cells with *unf*$^+$ and *unf*$^-$ phage (multiplicity of infection, about 7). Extracts were prepared as described by Warner et al. (47) and were assayed by the procedure of Wiberg et al. (50). One unit of enzyme activity is defined as the nanomoles of product produced per minute of reaction. Protein content of the extracts was measured using the Folin phenol procedure of Lowry et al. (21).

DNA synthesis. The synthesis of DNA in *unf*$^+$ phage- and *unf*$^-$ phage-infected *E. coli* B/5 cells was examined by measuring the incorporation of [^{3}H]thy-

midine into trichloroacetic acid-insoluble material. Subcultures (1.4 ml, 4×10^6 cells per ml) were infected with phage (multiplicity of infection, about 10; 0.5-ml suspension in M63 medium with Casamino Acids) 1 min after being transferred to preincubated (30°C) Erlenmeyer flasks, each containing 80 μg of tryptophan, 20 μg of 5-fluorodeoxyuridine, 50 μg of uracil, and 10 μg of thymidine. [^{3}H]thymidine (25 μl of a 1-mCi/ml solution; specific activity, 1.9 Ci/mmol) was added at 3 min after infection. Samples (50 μl) were transferred to Whatman GF/A glass-fiber disks at various times after infection. The disks were immersed individually in 10-ml volumes of cold 5% trichloroacetic acid, washed individually three times with 5-ml volumes of ethanol, dried on a warm hot plate, and counted in 10 ml of toluene–0.6% 2,5-diphenyloxazole scintillation fluid. The total radioactivity added to each subculture was determined by transferring a 50-μl sample to a Whatman GF/A disk, drying it, and counting it by liquid scintillation spectroscopy as described above.

RESULTS

Shutoff of host RNA synthesis in *unf*$^+$ phage- and *unf*$^-$ phage-infected B/5 cells. The rifampin-induced unfolding of the nucleoids of *E. coli* cells (27) suggested that ongoing transcription might be necessary to maintain the structure of folded genomes. If so, the nucleoids of cells infected with "unfoldase-defective" mutants of phage T4 might be expected to remain transcriptionally active. To test this possibility, we examined the template origin of RNA pulse-labeled with [^{3}H]uridine at various times after infection of *E. coli* B/5 cells with *unf*$^+$ and *unf*$^-$ phage by DNA-RNA hybridization, using purified *E. coli* and T4 DNA. To minimize the saturation of highly transcribed cistrons, DNA/RNA ratios of 10^3 and 5×10^2 for *E. coli* DNA and T4 DNA, respectively, were used. Under these conditions, the average efficiency of hybridization was 62.7%. The results of five experiments (Fig. 1) indicated that host RNA synthesis was, essentially, completely shut off by 10 min after infection of *E. coli* with the original unfoldase-defective mutant, *unf*39 (35). The shutoff of host RNA synthesis was significantly slower after infection of *E. coli* B/5 cells with *unf*39 than after infection with *unf*$^+$ phage, but nevertheless appeared to be about as complete by 10 min after infection. When the hybridization data were analyzed by the *t* distribu-

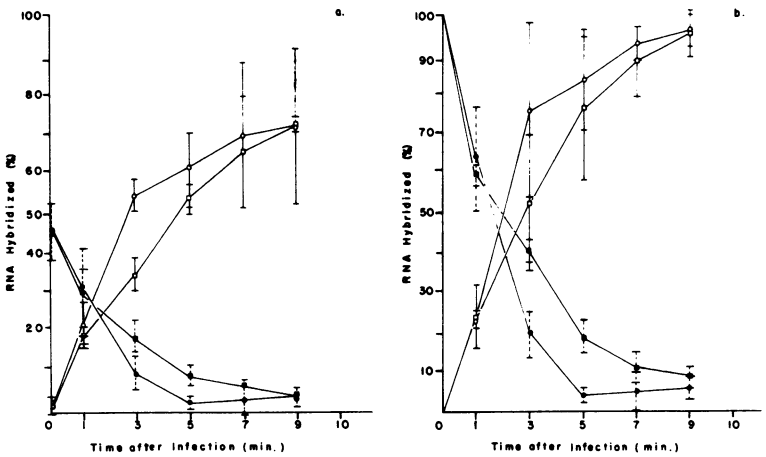

FIG. 1. *Switchover from host RNA synthesis to phage RNA synthesis in unf*$^+$ *phage- and unf*$^-$ *phage-infected E. coli B/5 cells. The proportion of* ^{3}*H-labeled RNA (1- or 2-min pulses; see text) added to each hybridization vial that hybridized with E. coli DNA (closed symbols) and with T4 DNA (open symbols) is plotted versus the time after infection (plotted at the midpoint of each pulse period). The values shown are the means of five experiments, and the bars (dashed for E. coli DNA, solid for T4 DNA) enclose ±1 standard deviation. The raw data are shown in (a), and normalized data are presented in (b), correcting for differences in hybridization efficiency to E. coli DNA and to T4 DNA (setting the value obtained for RNA pulse-labeled in uninfected cells as 100% for E. coli and the highest value obtained for T4 DNA after subtracting the E. coli component as 100% for T4). The unf*$^+$ *phage (circles) was T4D*$^+$ *in two experiments and amN82-rPT8-nd28x6 in three experiments (both shut off host RNA synthesis with the same efficiency [31]). The unf*$^-$ *phage (squares) was unf39x5 in two experiments and amN82-rPT8-nd28x6-unf39 in three experiments. One-percent hybridization averaged 138 cpm. DNA/RNA ratios were* 10^3 *and* 5×10^2 *for E. coli DNA and T4 DNA, respectively, in all experiments. The conditions of infection and pulse labeling, isolation of DNA and RNA, and hybridization were as described in the test. M63 medium (26) supplemented with 0.5% Casamino Acids was used as the growth medium in these experiments. The volume of preincubation mixture used in these experiments was 0.5 ml. The lowest efficiency of hybridization was 36%.*

tion data were analyzed by the t distribution, considering the values obtained for each time interval in *unf*$^+$ and *unf*$^-$ infections in a given experiment as paired variates, the differences between the means were statistically significant for the 2- to 4-min ($t = 15.2$, significant at $P = 0.001$), 4- to 6-min ($t = 7.3$, significant at $P = 0.01$), and 6- to 8-min ($t = 3.1$, significant at $P = 0.05$) pulse-labeling periods (4 df in all cases). The difference was not significant, however, for the 8- to 10-min pulse ($t = 2.2$).

Two of the experiments for which results are summarized in Fig. 1 compared the single mutant *unf* 39x5 (35) with wild-type T4D; the other three compared *am*N82-*r*PT8-*nd*28x6-*unf* 39 with *am*N82-*r*PT8-*nd*28x6 (DNA synthesis-, nuclear disruption-, and host DNA degradation-deficient [33]). Since the results appeared to be the same in both comparisons and since we have previously shown that *am*N82-*r*PT8-*nd*28x6 shuts off host RNA synthesis as efficiently as wild-type T4D (31), we believe that it is valid to pool the data from the two comparisons as we have done.

In addition to the slow shutoff of host RNA synthesis, the turn-on of T4 RNA synthesis was slower in *unf* 39 infections than in *unf*$^+$ infections (Fig. 1).

A key question is whether *unf* 39 is characteristic of *unf* mutants or whether it is somehow unique in behavior, perhaps leaky. We thus examined the shutoff of host transcription in *E. coli* B/5 cells infected (i) with nine independent *unf* mutants of spontaneous origin isolated in our laboratory, (ii) with the unfoldase-defective mutant *alc*1 (29, 37), and (iii) with mutant strain *r*NB2226, which is known to produce an altered 15,000-dalton RNA polymerase-binding polypeptide (H. R. Horvitz, D. I. Ratner, A. R. Poteete, and C. G. Goff, manuscript in preparation; C. G. Goff, personal communication), the putative product of the *unf*/*alc* gene (29).

The spontaneous *unf* mutants were isolated by taking advantage of the observation of Sirotkin et al. (29) that mutants of phage T4 that are capable of late transcription from cytosine-containing T4 DNA (*alc* mutants) (37) are sometimes *unf*$^-$ and that *alc* and *unf* are probably the same gene. Our results agree with those of Sirotkin et al. (29) in indicating that *alc* and *unf* are, very likely, the same gene. We analyzed nine *alc* mutants from a mass lysate plus nine independent *alc* mutants, all of spontaneous origin, and found all of them to be *unf*$^-$ by the pour test (35, 43). These were characterized as tight *alc* mutants by the temperature sensitivity criterion of Snyder and colleagues (29, 37) and by their inability when present with *am*E56 (gene 56, dCTPase-dUTPase) to grow on *N*-glycosidase-positive hosts after growth on dUTPase-deficient hosts (Duncan and Warner, manuscript in preparation). Four additional independent *alc* mutants of spontaneous origin were characterized as being slightly leaky by the above criteria. Of these, one was clearly intermediate between *unf*$^+$ and *unf*$^-$ by the pour-test criterion.

When pairwise complementation tests between *unf* 39 and these unfoldase-defective *alc* mutants were performed (mixed infections with strains carrying *r*PT8 and *nd*28x6 to eliminate nuclear disruption and host DNA degradation), the results were consistent with the proposal of Sirotkin et al. (29) that both *unf* and *alc* mutations occur in the same cistron. No complementation occurred. Dominance controls (mixed infections with the wild type and *unf* mutants) showed that the *unf* mutants are recessive to *unf*$^+$, supporting the validity of this interpretation of the pour-test complementation experiments. Subsequently, pour-test complementation experiments were done with *unf* 39 and the original *alc*1 and *alc*3 mutants studied by Snyder and colleagues (29, 37). The observed absence of complementation in these experiments provides further support for their proposal that *unf* and *alc* mutations identify the same gene. Finally, when *unf* 39 was combined with *am*E56 (gene 56, dCTPase-dUTPase) by recombination, it behaved as a tight *alc* mutant by the criteria of temperature sensitivity in *am su*$^-$ hosts and inability to grow on *N*-glycosidase-positive hosts after passage through dUTPase-deficient hosts.

In an attempt to identify *unf* mutants that fail to shut off host transcription, we screened the nine independent unfoldase-defective mutants of spontaneous origin by DNA-RNA hybridization, using RNA pulse-labeled with [^{3}H]uridine from 7 to 11 min after infection (at which time shutoff by *unf* 39 appears to be essentially complete; see Fig. 1) and purified *E. coli* and T4 DNAs. All nine of the spontaneous *unf* mutants had shut off host RNA synthesis to the same degree as *unf* 39 by 7 to 11 min after infection (data not shown). We selected the spontaneous mutant that yielded the highest percentage of RNA hybridization to *E. coli* DNA and compared the kinetics of the switch-over from host RNA synthesis to phage RNA synthesis with this mutant and *unf* 39. The results (Table 1) indicate that this spontaneous *unf* mutant shuts off host RNA synthesis with approximately the same kinetics as T4D$^+$.

Since all of the *unf*$^-$ mutants isolated in our laboratory shut off host RNA synthesis (albeit some of them more slowly than wild-type T4D) under our conditions, it became important to examine shutoff of host RNA synthesis under these conditions by the *alc*/*unf* mutants isolated

TABLE 1. *DNA-RNA hybridization analysis of the RNA synthesized at various times after infection of E. coli B/5 with unfoldase-defective and unfoldase-proficient T4 phage*

Labeled RNA from:[a]	Min post-infection	Hybridization to:[b]					
		Blank filter		*E. coli* DNA		T4 DNA	
		cpm bound	% of input cpm	cpm bound	% of input cpm	cpm bound	% of input cpm
Uninfected *E. coli* B/5		43	0.1	16,039	31.4	486	0.9
*am*N82-*r*PT8x2-*nd*28x6-infected B/5	2–4	58	0.1	1,565	3.1	14,692	29.1
	4–6	76	0.2	1,204	2.4	26,702	52.5
	8–10	58	0.1	564	1.1	30,235	57.2
	14–16	88	0.2	338	0.6	22,002	42.6
*am*N82-*r*PT8-*nd*28x6-*unf*39-infected B/5	2–4	65	0.1	6,474	12.0	21,382	39.7
	4–6	34	0.1	3,264	6.6	26,549	54.1
	8–10	51	0.1	559	1.1	35,027	66.8
	14–16	59	0.1	489	1.0	27,596	54.0
*am*N82-*r*PT8x2-*nd*28x6-*unf*S3-infected B/5[c]	2–4	81	0.2	1,962	3.7	27,720	52.3
	4–6	24	0.1	575	1.2	14,018	28.6
	8–10	63	0.1	443	0.9	26,338	51.1
	14–16	60	0.1	521	1.0	25,786	52.2

[a] Conditions of infection were as described in the text; the infections were done in Fraser glycerol medium (7). Unkilled cells were less than 1% at 5 min after infection in all cases.

[b] Each hybridization vial contained a filter charged with 100 μg of *E. coli* DNA, a filter charged with 50 μg of T4 DNA, a similarly processed blank filter, and about 1 μg of RNA in 3.0 ml of preincubation mixture.

[c] *unf*S3 is a spontaneous *unf/alc* mutant isolated during this study.

and studied by Snyder and colleagues (29, 37). Table 2 shows data comparing the template specificity of the RNA synthesized at various times after infection of *E. coli* B/5 with T4D$^+$, *unf*39x5, *am*E51-DD2-*alc*1 (*am*E51 is a gene 56 dCTPase-dUTPase-deficient mutation; DD2 is a double-deletion mutant missing all or parts of genes *r*IIA, *r*IIB, D1, and *den*B [37]), and *r*NB2226, a mutant strain producing an altered 15,000-dalton polypeptide (Horvitz et al., manuscript in preparation; Goff, personal communication) that is found associated with RNA polymerase after T4 infection (39) and is the putative product of the *alc* gene (29). Shutoff of host RNA synthesis was slower and, possibly, less complete after infection with *am*E51-DD2-*alc*1 than after infection with T4D$^+$, *r*NB2226, or *unf*39x5. However, it should be noted that, under the conditions used, *am*E51-DD2-*alc*1 adsorbed more slowly than the other strains. In particular, the slight increase in RNA hybridizing to *E. coli* DNA in the 14- to 16-min pulse-labeling period after infection with T4D$^+$ and *am*E51-DD2-*alc*1 may be due in part to the exponential growth of surviving cells (see footnote *a* of Table 2).

Kinetics of nuclear disruption, deoxyribonucleoside monophosphate kinase synthesis, and DNA synthesis in *unf*$^+$ phage- and *unf*$^-$ phage-infected B/5 cells. The kinetics of nuclear disruption in cells infected with T4D$^+$ and with *unf*39x5 were examined to see whether the failure to unfold the host nucleoid has any effect on pre-early gene expression. The results (Table 3) showed that *unf*$^+$ and *unf*$^-$ phage induce nuclear disruption with essentially the same, if not identical, kinetics. No cells showed nuclear disruption before 2 min after infection in either case. By 4 min after infection with phage of either genotype, over 50% of the cells had undergone nuclear disruption.

The kinetics of synthesis of the T4 early enzyme deoxyribonucleoside monophosphate kinase was also examined in *unf*$^+$ phage- and *unf*$^-$ phage-infected cells. The results (Fig. 2) give no indication of any delay in the induction of kinase synthesis by *unf*$^-$ phage, although the final level of kinase activity was consistently lower after infection with *unf*39x5. In *am*N82-*r*PT8-*nd*28x6-*unf*39 infections, the presence of *am*N82, which is known to result in increased levels of early enzyme synthesis (50), may partially compensate for the apparent effect of *unf*39 on final kinase activity levels.

The kinetics of DNA synthesis in *unf*$^+$ phage- and *unf*$^-$ phage-infected B/5 cells were examined by measuring the incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material in the presence of 5-fluorodeoxyuridine (to inhibit the thymidylate synthetases of the host

and phage). Somewhat surprisingly, the slow shutoff of host RNA synthesis and slow turn-on of phage RNA synthesis observed in *unf*⁻ phage-infected cells apparently has no measurable effect on the kinetics of DNA synthesis (Fig. 3).

DISCUSSION

Our results are consistent with the proposal of Sirotkin et al. (29) that the *unf* and *alc* mutations are located in the same gene. A conclusion as to whether this is indeed the case must await more definitive evidence, however. Our results also agree with their evidence indicating that the *alc/unf* gene(s) is involved in host RNA synthesis shutoff. Our results differ from those of Sirotkin et al. (29) as to the relative amounts of *E. coli*-specific and T4-specific RNA synthesis occurring subsequent to 8 to 10 min after infection with *alc/unf* mutants. We observed very little *E. coli*-specific RNA synthesis (0.3 to 5.0% of the total RNA synthesized during the 2-min labeling periods), whereas the data of Sirotkin et al. (29) suggested a large amount of *E. coli*-specific RNA synthesis (43 and 72% of the total pulse-labeled RNA) at 8 to 10 and 14 to 16 min postinfection. Clearly, these data can only be considered semiquantitative, since different RNA species hybridize with different efficiencies and the relative concentrations of different RNA species may change after infection with T4 phage. However, interpreted semiquantitatively, our data suggest that host RNA syn-

TABLE 2. *DNA-RNA hybridization analysis of the RNA synthesized at various times after infection of E. coli B/5 with T4D wild type and various unfoldase-defective mutants*

Labeled RNA from:[a]	3 Min postinfection	Hybridization to:[b]						
		Blank filter		*E. coli* DNA			T4 DNA	
		cpm bound	% of input cpm	cpm bound	% of input cpm[c]	% cpm *E. coli*-specific[d]	cpm bound	% of input cpm[c]
Uninfected *E. coli* B/5		18	0.3	2,011	34.2	(100)	36	0.3
T4D⁺-infected B/5	2–4	25	0.4	196	3.1	8.2	1,654	29.5
	4–6	10	0.2	51	0.8	1.5	1,590	29.2
	8–10	11	0.2	34	0.4	0.3	2,095	36.8
	14–16	18	0.3	59	0.7	1.2	2,103	36.6
*unf*39x5-infected B/5	2–4	17	0.3	331	6.0	16.8	3,851	73.3
	4–6	15	0.3	94	1.4	3.2	2,048	36.3
	8–10	18	0.3	44	0.5	0.6	3,514	61.5
	14–16	18	0.3	41	0.5	0.6	2,414	43.7
*am*E51-DD2-*alc*1-infected B/5	2–4	13	0.3	543	10.5	30.1	2,851	56.2
	4–6	16	0.3	230	4.0	10.9	3,963	73.0
	8–10	30	0.5	70	0.8	1.5	2,959	56.1
	14–16	19	0.3	133	2.1	5.0	3,321	59.6
*r*NB226-infected B/5[e]	2–4	20	0.4	193	3.3	8.8	2,836	53.5
	4–6	24	0.5	73	0.9	1.8	4,230	80.0
	8–10	19	0.3	67	0.9	1.8	2,303	41.9
	14–16	16	0.3	55	0.6	0.9	2,204	36.5

[a] Conditions of infection were as described in the text; Fraser glycerol medium (7) was the growth medium. Unkilled cells were less than 1% at 5 min after infection in all infections with T4D⁺, *unf*39x5, and rNB2226. With *am*E51-DD2-*alc*1, we observed 1 to 7% surviving cells at 5 min after infection under these conditions.

[b] Each hybridization vial contained a filter charged with 100 μg of *E. coli* DNA, a filter charged with 50 μg of T4 DNA, and a blank but similarly processed filter plus approximately 0.4 μg of RNA, all in 3 ml of preincubation mixture.

[c] Corrected for the binding to blank filters.

[d] Corrected for 0.3% nonspecific binding to heterologous DNA (based on the observed nonspecific binding of *E. coli* RNA to T4 DNA). These values must be considered only qualitative since they may be affected by differences in the concentrations of various species of RNA. In particular, saturation of the rRNA genes is known to occur even at the relatively high DNA/RNA ratios used.

[e] Not unfoldase-defective by our criteria but known to produce an altered 15,000-dalton RNA polymerase binding polypeptide (Horvitz et al., manuscript in preparation; Goff, personal communication), the putative product of the *unf/alc* gene (29).

TABLE 3. *Kinetics of nuclear disruption in unf⁺ phage- and unf⁻ phage-infected cells*

E. coli B/5 cells infected with:	Min postinfection	No. of cells scored	Cells with nuclear disruption present[a]	
			No.	%
*am*N82-*unf*39	10	139	90	65
*am*N82-*r*PT8-*nd*28x6	10	225	0	0
*am*N82-*r*PT8-*nd*28x6-*unf*39	10	151	0	0
T4D⁺	2	191	0	0
	4	474	316	67
	5	276	232	84
	6	279	236	85
	10	422	370	88
*unf*39x5	2	184	0	0
	4	587	303	52
	5	299	243	81
	6	295	228	77
	10	432	369	85

[a] Cells in which nuclear disruption had clearly occurred when scored by phase microscopy as described in the text. The rest of the cells were either ambiguous or did not appear disrupted.

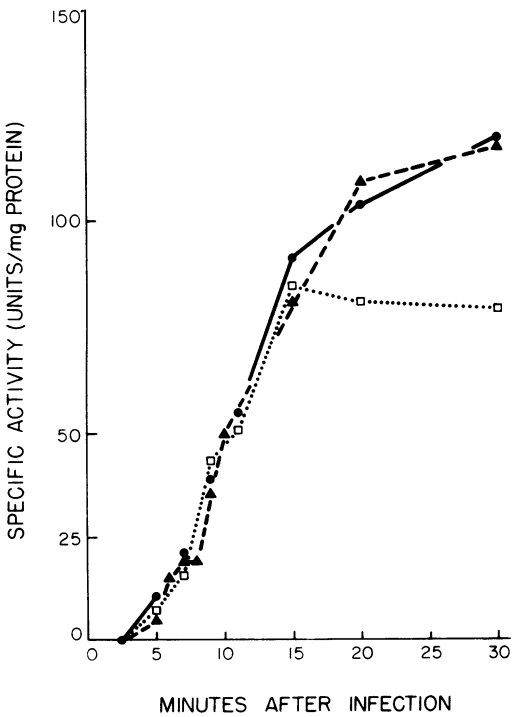

FIG. 2. *Kinetics of T4 deoxyribonucleoside monophosphate kinase synthesis in unf⁺ phage- and unf⁻ phage-infected E. coli B/5 cells. The infections, preparation of extracts, and assays were done as described in the text. One unit of enzyme activity yields 1 nmol of product per min at 30°C. E. coli B/5 cells were infected with T4D⁺ (●), unf39x5 (□), or amN82-rPT8-nd28x6-unf39 (▲).*

thesis shutoff is nearly complete by 8 to 10 min after infection, whereas the data of Sirotkin et al. suggest that little, if any, host RNA synthesis is shut off by the *alc/unf* mutants.

Our major reservation in accepting the results of Sirotkin et al. (29) is their observation that 31% of the RNA synthesized between 14 and 16 min after infection with T4 wild type is *E. coli* RNA. We (31; this paper; unpublished data) have never observed more than about 1% *E. coli* RNA in samples labeled later than 8 min after infection with wild-type T4D. Moreover, previous studies (in particular, see Nomura et al. [25], Landy and Spiegelman [19], and Kennell [13, 14]) failed to detect significant amounts of *E. coli*-specific RNA synthesis beyond about 5 min after infection. In all these studies, as in our experiments, pulse-labeled RNA was hybridized to both *E. coli* DNA and T4 DNA. Sirotkin et al. (29), on the other hand, have only carried out hybridization of their pulse-labeled RNA samples to *E. coli* DNA. We feel it is possible, therefore, that Sirotkin et al. have overestimated the amount of *E. coli*-specific RNA synthesis occurring at late times after infection with both wild-type T4 and *alc*1.

A second possible explanation for the discrepancy between the data of Sirotkin et al. (29) and our data is that the *alc*1 strains used were genetically nonidentical. Although the stock used in our studies was clonally derived from the inoculum provided by Snyder, it is possible that a partial suppressor mutation(s) (second-site reversion[s]) could have occurred. The stock did not contain any *alc*⁺ phage, but that would

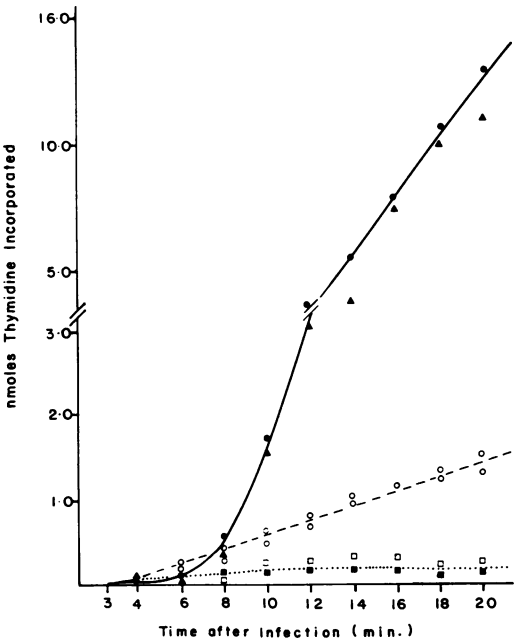

FIG. 3. *Kinetics of incorporation of [³H]thymidine into trichloroacetic acid-insoluble material in unf⁺ phage- and unf⁻ phage-infected E. coli B/5 cells in the presence of 5-fluorodeoxyuridine. The protocol was as described in the text; temperature of incubation was 30°C. Symbols:* (○) *uninfected B/5,* (●) *T4D⁺,* (▲) *unf39x5,* (□) *amN82-unf39,* (■) *amN82-rPT8-nd28x6-unf39.*

not exclude the possibility of partial revertants that could still be phenotypically *alc*⁻. It is clear from this study that *alc* mutants can be isolated that affect host RNA synthesis shutoff to varying degrees; e.g., contrast the apparent lack of effect of *unf* S3 (Table 1) with the effects of *unf* 39x5 and *alc*1 (Fig. 1 and Table 2). Moreover, it is apparent that these *alc/unf* mutants revert and that the *alc*⁻ phage are at a major selective disadvantage in competition with *alc*⁺ phage (except under conditions where only cytosine-containing phage DNA is synthesized). The occurrence of modifier mutations in these *alc/unf* mutant strains is, therefore, a possibility.

A third possibility is that both T4 wild type and the *unf/alc* mutants affect shutoff of host RNA synthesis differently under the different conditions (different host strains, media, etc.) used in the two studies. The experiments of Sirotkin et al. were done with *E. coli* strain B^E growing in M9 medium. Ours were done with *E. coli* strain B/5 in Fraser glycerol medium (7) and in M63 medium (26) supplemented with Casamino Acids (0.5%). Our results were the same in both media. The major difference in the growth conditions used by Sirotkin et al. and in our experiments would appear to be the presence or absence of the Casamino Acids supplement. What effect this difference might have on host RNA synthesis shutoff, if any, is unclear.

Despite the discrepancy between our results and those of Sirotkin et al. (29) discussed above, it is clear that some *alc/unf* mutants are altered in the shutoff of the synthesis of at least some species of host RNA. R. Buckland and A. Travers (A. Travers, personal communication) have shown that cells infected with *unf* 39x5 synthesize several times as much rRNA between 5 and 10 min after infection as cells infected with wild-type T4. Moreover, Buckland and Travers (personal communication) have shown that the RNA polymerase isolated from *unf* 39x5-infected cells is more sensitive to inhibition of rRNA synthesis by guanosine 5′-diphosphate 3′-diphosphate (42) than RNA polymerase isolated from T4⁺-infected cells. Travers (42) has presented evidence that the *E. coli* RNA polymerase holoenzyme can exist in at least two different conformations, one with a high affinity for rRNA promoters and one with a low affinity, and that guanosine tetraphosphate stabilizes the enzyme in the low-affinity state. Baralle and Travers (1) showed that T4 infection has the same effect on RNA polymerase with respect to rRNA synthesis as guanosine tetraphosphate. The addition of guanosine tetraphosphate does not decrease the fraction of rRNA synthesized by the enzyme from T4-infected cells, in contrast to its effect on the enzyme from uninfected cells. Baralle and Travers (1) proposed that one of the four T4 proteins noncovalently attached to the *E. coli* RNA polymerase (39) stabilizes the enzyme in the rRNA low-affinity conformation. Sirotkin et al. (29) reported that RNA polymerase from *alc*-infected cells is missing one of these proteins, the 15,000-dalton polypeptide. If *alc* and *unf* are, in fact, the same gene and code for this 15,000-dalton polypeptide, the results of Travers with the RNA polymerase from *unf*-39x5-infected cells fits very nicely with the proposal of Baralle and Travers.

The effect of the *alc/unf* mutations on the shutoff of host mRNA synthesis is less clear. E. M. Kutter (personal communication) has preliminary results that indicate that when the RNA extracted from cells at 25 min after infection at 37°C with *alc*2 (34) is used in an in vitro protein synthesis system and the proteins synthesized are analyzed by polyacrylamide gel electrophoresis, the pattern observed appears identical to that obtained in an experiment using a mixture of equal amounts of RNA from uninfected cells and RNA from T4⁺-infected cells. Her results support the data of Sirotkin et al. (29), which suggest that *alc/unf* mutants fail to

shut off host mRNA synthesis. The results presented in this paper, on the other hand, would necessitate that most mRNA synthesis be shut off by 8 to 10 min after infection with *unf* 39 and *alc*1. Whereas saturation of rRNA cistrons will occur even at the high DNA/RNA ratios used (e.g., 1,000/1), saturation with mRNA's would not be expected. Thus, the continued synthesis of host mRNA should have been readily detectable. We thus conclude that most, if not all, host mRNA synthesis is shut off after infection of *E. coli* B/5 with *unf*$^-$ T4 phage.

R. Buckland and A. Travers (Travers, personal communication) have observed that the RNA polymerase from *unf* 39x5-infected cells fails to bind the restriction fragment of phage ϕ80 psu$^+_{III}$ carrying the su$^+_{III}$ tRNA promoter and some ϕ80 promoter fragments, like the RNA polymerase from T4$^+$-infected cells and unlike the RNA polymerase from uninfected *E. coli* cells. Nomura et al. (25) have shown that the shutoff of host stable RNA responds differently than the shutoff of mRNA to chloramphenicol and varying multiplicity of T4 infection. Mailhammer et al. (23) observed a reduction of *E. coli* β-galactosidase synthesis in a coupled in vitro protein-synthesizing system when RNA polymerase from T4-infected cells was used. On the basis of reconstruction experiments, they concluded that "modification of the α-subunit of the RNA polymerase is sufficient for inhibition of host transcription." Their results thus strongly indicate a role for the ADP-ribosylation of the α-subunit of RNA polymerase (9) in the shutoff of host mRNA synthesis. In addition, Stevens and Rhoton (40) have evidence indicating that the 10,000-dalton polypeptide found associated with RNA polymerase after T4 infection inactivates or alters the sigma subunit. Assuming that sigma is required for in vivo protein synthesis, this 10,000-dalton protein would be expected to inhibit RNA synthesis. Thus, present evidence implicates ADP-ribosylation of the α-subunit of RNA polymerase, the 10,000-dalton RNA polymerase-associated protein, and the product(s) of the *alc/unf* gene(s) (apparently the 15,000-dalton, RNA polymerase-associated protein) in the T4-induced shutoff of host transcription. Clearly, further studies are necessary to elucidate the roles of each of these putative host transcription shutoff factors. Moreover, it is now totally clear (13, 14, 29, 35) that host protein synthesis is also shut off after T4 infection by a separate mechanism acting at the level of translation.

Interestingly, the slow shutoff of host RNA synthesis after infection of *E. coli* with *unf* 39x5 is paralleled by a slow turn-on of T4 RNA synthesis, such that the total amount of RNA synthesis per cell appears to remain constant regardless of whether the infecting phage is T4D$^+$ or *unf* 39x5. We (35) have previously shown that this is not due to slow adsorption. Moreover, when the kinetics of DNA synthesis, T4 deoxyribonucleoside monophosphate kinase synthesis, and nuclear disruption were compared for T4D$^+$- and *unf* 39x5-infected cells, no measurable differences were observed. This suggests that some T4 early mRNA's, like most of the T4 early proteins (30), are synthesized in excess, at least under optimal growth conditions.

ACKNOWLEDGMENTS

This research was supported by National Science Foundation grant PCM76-01841.

We thank A. Travers, C. G. Goff, and E. M. Kutter for communicating results before publication, and B. Duncan, C. G. Goff, L. Snyder, and H. R. Warner for providing mutant strains used in the study.

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