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On the Direction of Reading of Bacteriophage T4 Gene 43 (Deoxyribonucleic Acid Polymerase)

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Amber (*am*) mutants of the two closely linked sites, B22 and C125, in bacteriophage T4 gene 43 [deoxyribonucleic acid (DNA) polymerase] synthesize in the nonpermissive (*su*$^-$) *Escherichia coli* host gene 43 products which are devoid of DNA polymerase activity, but which retain a 3'-exonuclease activity. Diethylaminoethylcellulose chromatographic analysis of DNA polymerase and deoxyribonuclease activities from extracts of *su*$^-$ cells infected with single- and double-*am* mutants of T4 gene 43 showed that the exonuclease activity which is observed with *am*B22 is not seen with double mutants carrying, in addition to *am*B22, *am* mutations which map to the clockwise side of the B22 site on the circular genetic map of T4. Similarly, *am* mutations which map to the clockwise side of the C125 site abolish the exonuclease activity which is observed with an *am* mutant (*am*E4335) of this site. It was concluded that in these double mutants termination signals to the clockwise side of *am*B22 and *am*E4335 are encountered before the *am*B22 and *am*E4335 signals during translation of the messenger ribonucleic acid from T4 gene 43. Thus, it seems that the T4 DNA polymerase is synthesized in vivo in a direction which corresponds to a counterclockwise reading of gene 43.

The product of bacteriophage T4 gene 43 has been shown to have two activities: a deoxyribonucleic acid (DNA)-synthesizing activity (DNA polymerase) and an exonucleolytic activity which releases 5'-mononucleotides from the 3'-hydroxyl terminus of heat-denatured or partially digested DNA (3'-exonuclease) (9, 15, 33). Several amber (*am*) mutations of T4 gene 43 have been isolated (R. S. Edgar, *personal communication*) and mapped (2, 11). They all fail to propagate in nonpermissive (*su*$^-$) *Escherichia coli* hosts, suggesting that the gene 43 DNA polymerase is essential for normal phage growth (13, 33). Nossal has shown that in the *su*$^-$ host the gene 43 mutant *am*B22 synthesizes a gene 43 product which does not have any measurable polymerase activity, but which does act as a 3'-exonuclease (25). Recently, it was shown that gene 43 *am* mutants which map at the C125 site, which is closely linked to the B22 site, also induce an exonuclease activity (26). On the, other hand, gene 43 *am* mutants which map to both sides of the B22 and C125 sites fail to induce gene 43 products with nuclease activity. The nuclease activities induced by *am* mutants of the B22 and C125 sites are distinguishable from the wild-type gene 43 polymerase-associated nuclease by their different elution properties in diethylaminoethyl (DEAE)-cellulose chromatography.

We have also observed the altered nuclease activities which are synthesized by *am* mutants of the B22 and C125 sites. In this study, we used such mutants to determine the direction of translation for the gene 43 DNA polymerase in vivo. We tested the effects of other gene 43 *am* mutations on the abilities of *am*B22 (B22 site) and *am*E4335 (C125 site) to produce the altered exonucleases. It was found that these nucleases were not synthesized in *su*$^-$ cells which were infected with gene 43 double-*am* mutants carrying, in addition to *am*B22 or *am*E4335, *am* mutations which map to the clockwise side of the B22 and C125 sites on the circular genetic map of T4. This probably means that in these double mutants termination signals to the clockwise side of *am*B22 or *am*E4335 are encountered before the *am*B22 or *am*E4335 signal during translation of the messenger ribonucleic acid from gene 43. We conclude that the T4 DNA polymerase is synthesized in a direction which corresponds to a counterclockwise reading of gene 43. The effects of *am* mutations which map to the counterclockwise side of the B22 and C125 sites on the nuclease activities that are induced by *am*B22 and *am*E4335 could not be tested. Only one gene 43 *am* site is known which maps to that side of the B22 and C125 sites, and it seems, from our attempts to isolate the required

double-*am* mutants, that these double mutants are not viable.

MATERIALS AND METHODS

Materials. DEAE-cellulose (Cellex D with an exchange capacity of 0.60–0.79 meq/g) was a product of BioRad Laboratories, Richmond, Calif. Salmon sperm DNA was purchased from Calbiochem, Los Angeles, Calif. Pancreatic deoxyribonuclease I was purchased from Worthington Biochemical Corp., Freehold, N.J. Phenylmethylsulfonyl fluoride (PMSF), thymidine-*methyl-^3H* (^{3}H-TdR), and tetralithium thymidine-5′-triphosphate-*methyl-^3H* (^{3}H-TTP) were purchased from Schwarz/Mann, Orangeburg, N.Y. Bacteriological grade A-305 alumina was purchased from Alcoa, Bauxite, Ark.

Phage and bacterial strains. All T4 *am* mutants used were a gift from J. Speyer. The gene 43 *am* mutants are listed in Table 1. Double-*am* mutants were constructed by genetic recombination, and their composition was checked both by (i) their inability to yield wild-type recombinants in crosses with the parental single *am* mutants and (ii) by crosses with wild-type phage which yielded the original single *am* mutants.

E. coli CR63 (*su*$^+$1, *ser*) and K110 *str*^{r} (*su*$^+$3, *tyr*) were used as the permissive hosts for T4 *am* mutants. The nonpermissive (*su*$^-$) hosts were *E. coli* S/6 *str*^{r} and W3110 *pol* A_1^+ *str*^{r}. The *str*^{r} strains are resistant to 200 μg of streptomycin per ml. *E. coli* K110 (λ), the streptomycin-sensitive ancestor of K110 *str*^{r}, was obtained from N. Zinder via J. Speyer. [It is strain H12R8a of Garen et al. (14).] *E. coli* CR63 and S/6 were from R. S. Edgar. *E. coli* S/6 is a derivative of *E. coli* B (10). *E. coli* W3110 *pol* A_1^+ *str*^{r} was derived from *E. coli* W3110 *pol* A_1 *str*^{r} and is resistant to 0.04% (w/v) methylmethanesulfonate. The *pol* A_1 strain carries an *am* mutation in the structural gene of a DNA polymerase (8, 16, 20) which is used in the repair of ultraviolet-damaged *E. coli* and phage T4 DNA in vivo (21, 24, 28). The *pol* A_1^+ *str*^{r} derivative has normal levels of this polymerase. The *E. coli* *pol* A_1 parental strain was obtained from J. Cairns. *E. coli* B_3 *thy*$^-$ which was used to prepare ^{3}H-labeled DNA was obtained from M. Oishi.

Growth conditions. Cells were grown at 30 C in a modified M9 medium (1) containing 42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.5 mM NaCl, 18.6 mM NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.01 mM $FeCl_3 \cdot 6H_2O$, 0.25% (w/v) Difco vitamin-free Casamino Acids, 0.4% (w/v) glucose, 20 μg of L-tryptophan per ml, and 1 μg of thiamine hydrochloride per ml. In experiments involving the infection of *E. coli* W3110 *pol* A_1^+ *str*^{r} with T4 *am* mutants, this medium was supplemented with 5 μg of thymine per ml, and the final concentration of glucose was 1% instead of 0.4%. One-liter cultures of *E. coli* W3110 *pol* A_1^+ were started from small inocula of stationary-phase cells. The size of an inoculum was adjusted such that growth by aeration at 30 C for 12 to 15 hr (overnight) yielded a log-phase culture with a cell density of 5×10^8 to 8×10^8 cells per ml. L-Tryptophan was added to the 1-liter culture to give a final concentration of 30 μg per ml, and phage was then added to give a multiplicity of infection of 5. The infection mixture was aerated at 30 C in a rotary shaker. At 60 min after infection, the culture was poured over ice cubes and the cells were harvested by centrifugation at $4{,}100 \times g$ for 15 min at 4 C. The cells were then suspended in 60 ml of 50 mM tris(hydroxymethyl)aminomethane (Tris), *p*H 7.5, centrifuged at $3{,}200 \times g$ for 5 min at 4 C, and the pellet was used to prepare the crude extracts.

Infection was monitored by the shut-off of host DNA synthesis as measured by thymidine incorporation. Samples (0.1 ml) of culture were withdrawn at various times after infection and incubated with 0.5 μCi of ^{3}H-TdR (6,000 mCi/mmole) for 3 min at room temperature. Incorporation was stopped with cold 10% (w/v) trichloroacetic acid. A decrease in the rate of ^{3}H-TdR incorporation to 10 to 15% the level measured for uninfected cells was considered indicative of successful infection.

For labeling *E. coli* DNA, B_3 *thy*$^-$ cells were grown at 30 C in the modified M9 medium supplemented with 4 μg of ^{3}H-TdR per ml (24.2 mCi/mmole). A 1-liter culture was grown from an initial density of 7×10^4 cells per ml, and the cells were harvested when the culture reached a density of 2×10^9 cells per ml as determined by microscope cell counts. Viable counts were also determined and showed no measurable loss of viability under these growth conditions.

Other media and methods for growth of bacterial and phage strains and for phage assays were as described by Steinberg and Edgar (29).

Phage crosses. A mid-log phase culture of *E. coli* CR63 grown in the modified M9 medium was diluted in fresh medium to a concentration of 1×10^8 cells per ml. The cells (0.5 ml total volume) were infected with a mixture of the parental phages at a multiplicity of infection of 5 each and aerated gently at 30 C. At 10 min after infection, T4 antiserum (final concentration $K = 1/\text{min}$) was added to remove unadsorbed phage. At 20 min after infection, the mixture was diluted 1,000-fold in fresh medium and aerated at 30 C for 40 min and then chloroform was added to lyse the cells. *E. coli* CR63 was used to assay infective centers (at 20 min after infection) and total phage yield. *E. coli* S/6 *str*^{r} was used to assay for wild-type recombinants.

DEAE-cellulose chromatography of crude extracts. The cell pellet from a 1-liter culture was ground for 15 min with 10 g of alumina and 12 ml of extraction

TABLE 1. T4 *Gene 43 am mutants*

Site[a]	Representative mutant used
E4302	*am*E4302
B22	*am*B22
C125	*am*E4335[b]
E4317	*am*E4317
E4309	*am*E4309
E4322	*am*E4322

[a] E. F. Allen, I. Albrecht, and J. Drake, 1970.
[b] J. Drake, 1971.

buffer (25) containing 50 mM Tris (pH 7.5), 25% (v/v) glycerol, 10 mM $MgCl_2$, 0.5 mM 2-mercaptoethanol, 1.0 mM ethylenediaminetetraacetic acid, and 0.56 mM PMSF. The ground slurry was then centrifuged at 30,000 × g for 20 min and then at 102,000 × g for 60 min. The supernatant fluid (9 ml) was applied to a DEAE-cellulose column (0.9 by 20 cm) equilibrated with 50 mM Tris (pH 7.5) containing 15% (v/v) glycerol, 0.56 mM PMSF, and 10 mM 2-mercaptoethanol. The column was then washed with 9 ml of the same buffer and eluted at a flow rate of 0.5 ml/min with 100 ml of a linear 0 to 0.5 M gradient of NaCl prepared in the same buffer. Fractions (2 ml) were collected. The salt gradient was measured with a model 31 conductivity bridge (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio).

Enzyme assays. DNA polymerase was assayed at 30 C by the method of Goulian et al. (15) with alkaline-denatured (31) salmon sperm DNA as priming template. The specific activity of ^{3}H-TTP in the reaction mixture was 20.1 mCi/mmole. A unit of enzyme was defined as the incorporation of 10 nmoles of ^{3}H-TTP in 60 min at 30 C. In assays where the effect of 0.11 mM *p*-chloromercuribenzoate (pCMB) was studied, 2-mercaptoethanol and sodium fluoride were omitted from the reaction mixture.

The source of substrate for deoxyribonuclease assays was tritium-labeled *E. coli* B_3 *thy*$^-$ DNA which was extracted and purified by a modified method of Kay et al. (7, 19). This native ^{3}H-DNA preparation was heat-denatured to render it an active substrate for the nucleases of interest. The native ^{3}H-DNA preparation was heated for 10 min at 100 C and chilled to 4 C, and the DNA was precipitated by the addition of 0.1 volume of cold 70% (w/v) trichloroacetic acid. The resulting precipitate was collected by centrifugation and dissolved in 3 volumes of 2.0 mM $NaHCO_3$-20.0 mM glutathione, the pH of the solution being adjusted to 8 to 9 by the addition of Na_2CO_3. Deoxyribose content of the ^{3}H-DNA was determined by the method of Burton (5). Prior to assay, this preparation was further treated with pancreatic deoxyribonuclease I (1.3 ng/μg of DNA-phosphate) at 37 C for 60 min. We found that this led to an almost fivefold increase in activity measured on *am*B22-induced nuclease peak fractions while increasing the soluble background radioactivity only 1%.

The nuclease assay mixture (0.50 ml) contained 62 mM Tris (pH 8.8), 13 mM $MgCl_2$, 2.3 nmoles of DNA-phosphate (about 12,000 ^{3}H-count/min), and 0.20 ml of chromatography fraction. The assay was carried out at 30 C. After 60 min of incubation, 0.1 ml of carrier bovine serum albumin (700 μg per ml) was added, followed by 0.1 ml of cold 44% (w/v) trichloroacetic acid. The precipitate was removed by centrifugation at 12,000 × g for 10 min, and 0.2 ml of the supernatant fluid was counted in 10 ml of 1,4-dioxane-based scintillator. A unit was defined as the solubilization of 10 nmoles of total nucleotide under the conditions of assay.

Glucose-6-phosphate dehydrogenase (G6PD) was assayed by the method of Kornberg and Horecker (22).

Protein. Samples of crude extracts or column fractions were dialyzed against 50 mM Tris, pH 7.5, and assayed for protein concentration by the method of Lowry et al. (23).

RESULTS

Genetic map order of gene 43 am mutations. Figure 1 shows the orientation of gene 43 on the T4 genetic map and the order of *am* sites which are relevant to this study. The order of sites was determined by Allen et al. (2) using two-factor crosses and is confirmed here by the results of three-factor crosses. Some of the results of this analysis are shown in Table 2. The relative order of the closely linked B22 and C125 sites is ambiguous, although the results suggest that C125 is to the clockwise side of B22.

DEAE-cellulose chromatography of DNA polymerase and deoxyribonuclease. Nossal (25) and Nossal and Hershfield (26) screened several *am* mutants of gene 43 for their ability to synthesize the polymerase-associated 3′-exonuclease activity in the *su*$^-$ host. They found such activity in extracts of cells infected with *am* mutants of the B22 and C125 sites but not with *am* mutants of other gene 43 sites. In the following experiments, we confirm these observations and also show that the nucleases which are induced by *am*B22 and *am*E4335 are abolished by *am* mutations that map to the clockwise side of the B22 and C125 sites.

Figure 2 shows the results of DEAE-cellulose chromatography of DNA polymerase and de-

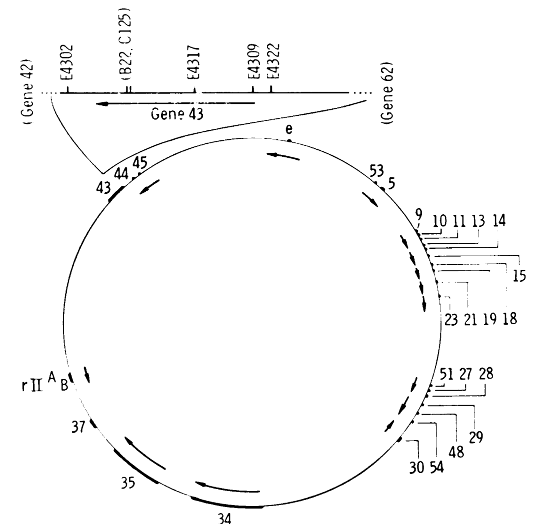

FIG. 1. *Circular linkage map of bacteriophage T4 [as reported by Edgar and Wood (12)] showing genes for which a direction of transcription or translation (arrows) has been determined [for a review, see Calendar (6)]. Enlargement of gene 43 shows the relative positions of am mutations used in our experiments.*

TABLE 2. *Ordering gene 43 am mutants by three-factor crosses*[a]

Cross	Recombination frequency (% wild type)	Order deduced				
*am*E4302 × *am*B22	1.25					
*am*E4322 × *am*B22	2.08					
*am*E4302 × *am*B22-*am*E4322	1.06					
*am*B22 × *am*E4302-*am*E4322	0.37	*am*E4302	*am*B22			*am*E4322
*am*E4309 × *am*B22	1.76					
*am*E4302 × *am*B22-*am*E4309	1.35	*am*E4302	*am*B22		*am*E4309	
*am*E4309 × *am*E4322	0.47					
*am*E4309 × *am*B22-*am*E4322	0.20					
*am*E4309 × *am*E4302-*am*E4322	0.19		*am*B22		*am*E4309	*am*E4322
*am*E4335 × *am*B22	0.0064					
*am*E4335 × *am*E4309	1.27					
*am*E4335 × *am*B22-*am*E4309	0.0028					
*am*E4335 × *am*E4322	2.29					
*am*E4335 × *am*B22-*am*E4322	0.0019		*am*B22	*am*E4335	*am*E4309	*am*E4322
Summary		*am*E4302	*am*B22	*am*E4335	*am*E4309	*am*E4322

[a] The results shown were obtained in a single experiment. Burst sizes obtained in this experiment ranged between 150 to 260 viable phage per infective center.

oxyribonuclease activities in an extract of cells infected with T4 carrying wild-type gene 43. The T4 DNA polymerase and the associated 3′-exonuclease activities appeared in one peak (0.11 M NaCl). The T4-induced and the host DNA polymerase activities were incompletely resolved, but they could be distinguished by their different sensitivities to pCMB. The T4 DNA polymerase activity was completely inhibited in the presence of 0.11 mM pCMB, but a small amount of activity corresponding to host enzyme (0.18 M NaCl) remained. The host G6PD, which eluted at a point roughly midway between the peaks of T4 and host DNA polymerase activities (0.16 M NaCl), served as a marker in these experiments.

Extracts of cells infected with various single-*am* and double-*am* mutants of T4 gene 43 were also analyzed. Some results are shown in Fig. 3. None of the mutants examined showed the wild-type T4 DNA polymerase peak. However, a new peak of nuclease activity, which eluted at about 0.05 M NaCl, was observed with *am*B22 (Fig. 3B). Both *am*E4322 and *am*B22-*am*E4322 lack this activity (Fig. 3C and D). Host DNA polymerase was detected in all extracts. The peak of nuclease activity, which eluted at about 0.27 M NaCl, was observed with uninfected and all T4-infected cells. Part of this nuclease activity in extracts of infected cells may be due to the T4-induced oligonucleotide diesterase (exonuclease A) which was described by Oleson and Koerner (27).

In the experiments for Fig. 3, we observed a peak of nuclease activity which eluted at about the same position as the wild-type polymerase-exonuclease. This activity is probably not a product of gene 43 since it was observed with all the single- and double-*am* mutants of gene 43 which we have examined. However, in experiments where extracts containing less than 1 mg/ml were used for chromatography, this nuclease activity was not observed, whereas a prominent peak of B22 nuclease activity remained. This is shown in Fig. 4 where the difference between *am*B22 and *am*B22-*am*E4322 appeared more pronounced due to the decrease in exonuclease activity eluting at about 0.11 M salt. It should be noted that no DNA polymerase activity was detected in this experiment and in other experiments (results not shown) where small amounts of extract were used in the chromatography.

The results in Fig. 3 and 4 show that the chromatographic pattern of nuclease activity obtained with *am*B22-*am*E4322 clearly resembles that of *am*E4322 but not that of *am*B22. Thus, it seems that *am* mutations which map to the clockwise side of *am*B22 abolish the effect of the *am*B22 mutation.

Experiments with *am*E4335 (C125 site) are described in Fig. 5. It was observed that a chromatographically altered nuclease activity is produced in su^- cells after infection with *am*E4335 (Fig. 5A) but not with the double mutants *am*E4335-*am*E4309 (Fig. 5B) and *am*E4335-*am*E4322 (Fig. 5C). Both *am*E4309 and *am*E4322 map to the clockwise side of *am*E4335 (Table 2). In another experiment (results not shown), the double mutant *am*B22-*am*E4309 behaved

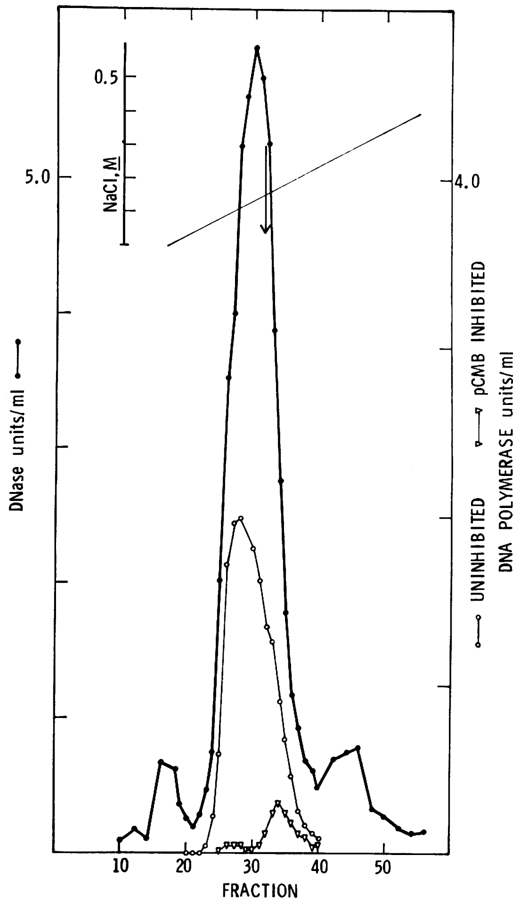

FIG. 2. *DEAE-cellulose chromatography of DNA polymerase and deoxyribonuclease activities in an extract of E. coli W3110 pol* A_1^+ *str*^{r} *cells infected with T4 amN82 (gene 44, function unknown). Nine milliliters of crude extract containing 27 mg of protein was chromatographed. Arrow indicates the peak of G6PD activity.*

similarly to *am*E4309 (Fig. 5D) and *am*E4335-*am*E4309 (Fig. 5B).

In all experiments involving single *am* mutants of the B22 and C125 sites, we observed still another nuclease activity which eluted at a point immediately preceeding the salt-gradient elution (Fig. 3 and 5). This activity is probably related to the *am*B22 and *am*E4335 products since it was not observed with other single *am* mutants of gene 43 and was also absent in cells infected with the double *am* mutants which lacked the *am*B22 and *am*E4335 nucleases.

Table 3 summarizes the results of DNA polymerase assays which were performed in the presence and absence of pCMB on the fractions collected in the chromatography experiments described above. As can be seen, extracts from uninfected as well as T4 *am*-infected cells gave polymerase activities which were largely insensitive to inhibition by pCMB. This probably means that in our experiments all of the polymerase activity which was detected was host-derived.

Nonviability of amE4302-amB22 and amE4302-amE4335 double mutants. Several attempts at constructing the double mutants, *am*E4302-*am*B22 and *am*E4302-*am*E4335, have been unsuccessful. The E4302 site is the only known *am* site which maps to the counterclockwise side of the B22 and C125 sites, and the chromatographic pattern of deoxyribonuclease activity in extracts of su^- cells infected with *am*E4302 is identical to that of *am*E4322 in Fig. 3C. If reading of gene 43 is indeed counterclockwise, as the results in Fig. 3, 4, and 5 suggest, then the expected effect of premature termination of protein synthesis in su^- cells infected with *am*E4302-*am*B22 or *am*E4302-*am*E4335 would be production of the activities which are characteristic of the B22 and C125 sites.

Amber mutants of the E4302 site can be propagated only in su^+ hosts which insert serine (su^+1) or tyrosine (su^+3 and su^+4) in response to the *am* codon (2), although growth of these mutants on these hosts is temperature sensitive (*unpublished data*). Several crosses between *am*E4302 and *am*B22 yielded 1.6 to 2.0% wild-type recombinants but did not yield any double mutant recombinants among 336, 500, and 288 progeny produced on a su^+1 host in three independent attempts, or among 1,683 progeny produced on a su^+3 host in one attempt. Attempts to isolate a double *am* mutant carrying mutations at the E4302 and C125 sites also failed. In this case, a cross between *am*E4302 and *am*E4335 which yielded 2.2% wild-type recombinants did not yield any double-mutant recombinants among 600 progeny produced on a su^+1 host or among 920 progeny produced on a su^+3 host. We suspect, therefore, that *am*E4302-*am*B22 and *am*E4302-*am*E4335 cannot propagate on any of the known *am* suppressors.

DISCUSSION

The DNA polymerase coded for by bacteriophage T4 gene 43 (9, 33) is active both as a DNA-synthesizing enzyme and as a 3'-exonuclease which releases 5'-mononucleotides from 3'-hydroxyl termini of denatured or partially digested DNA substrates (15). Apparently, the DNA-synthesizing activity is essential for phage growth since *am* mutations of gene 43 which result in the loss of this activity also lead to lethality (13, 33). On the other hand, the significance of the exonucleolytic activity is less clear. Nossal (25) and Nossal and Hershfield (26) have shown

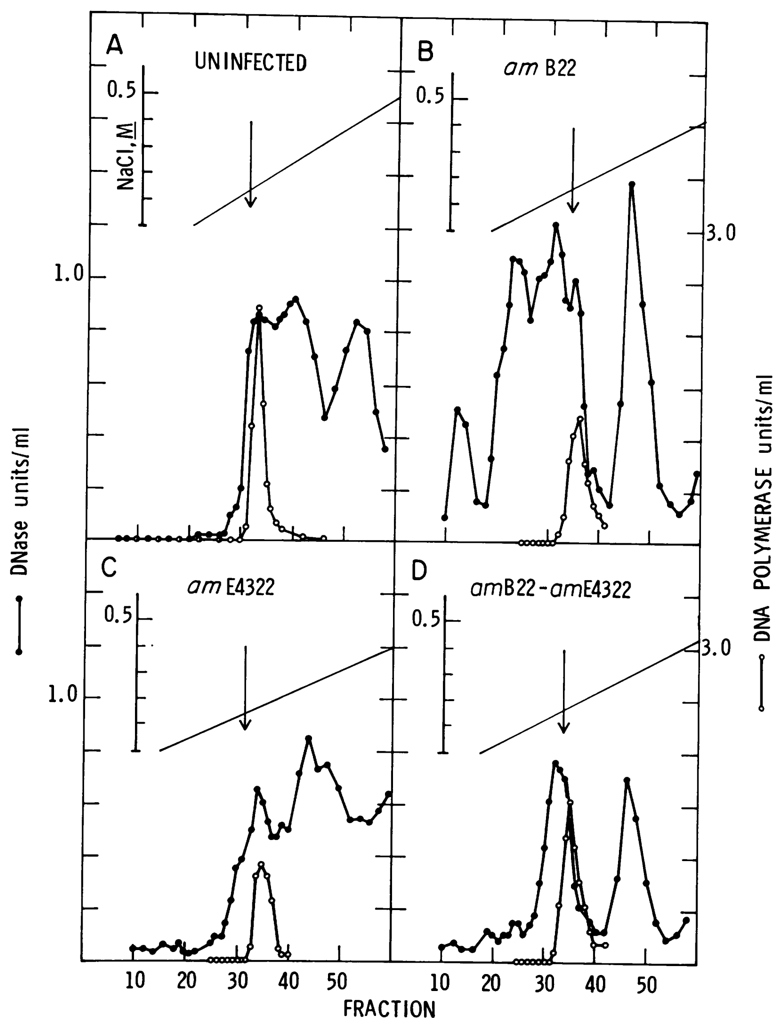

FIG. 3. *DEAE-cellulose chromatography of DNA polymerase and deoxyribonuclease activities in extracts of E. coli W3110 pol* A_1^+ *str*^{r} *cells infected with am mutants of T4 gene 43. In all experiments, 9 ml of crude extract was chromatographed. The following amounts of total protein were applied to the columns: uninfected cells, 31.6 mg; amB22, 27 mg; amE4322, 24.3 mg; amB22-amE4322, 30.8 mg. Arrow indicates the peak of G6PD activity.*

and we have confirmed that the gene 43 products produced in *su*$^-$ cells infected with gene 43 *am* mutants of the B22 and C125 sites are devoid of polymerase activity but have in vitro exonuclease activities which are chromatographically identical to each other but distinct from the wild-type polymerase-associated 3′-exonuclease. Since the effect of *am* mutations is the premature termination of protein synthesis at the site of the *am* (UAG) codon (3, 4), these gene 43 products presumably consist of N-terminal fragments of the wild-type DNA polymerase molecule (30).

We used the mutants *am* B22 and *am*E4335 in studies on the direction of reading of T4 gene 43 in vivo. In these studies, we confirmed the relative order of certain *am* mutations in gene 43 by three-factor crosses and then used certain double-*am* mutants of gene 43 to test the effects of second *am* mutations on the nuclease activities which are induced by *am* mutants of the B22 and C125 sites. In the double-*am* mutants we examined, it was observed that *am* mutations which map to the clockwise side of *am*B22 and *am*E4335 effectively abolished the potential of *am*B22 and *am*E4335 to produce their characteristic exonuclease activities. Thus, it appears that *am* termination signals clockwise to *am*B22 and *am*E4335 are encountered before the *am*B22 and

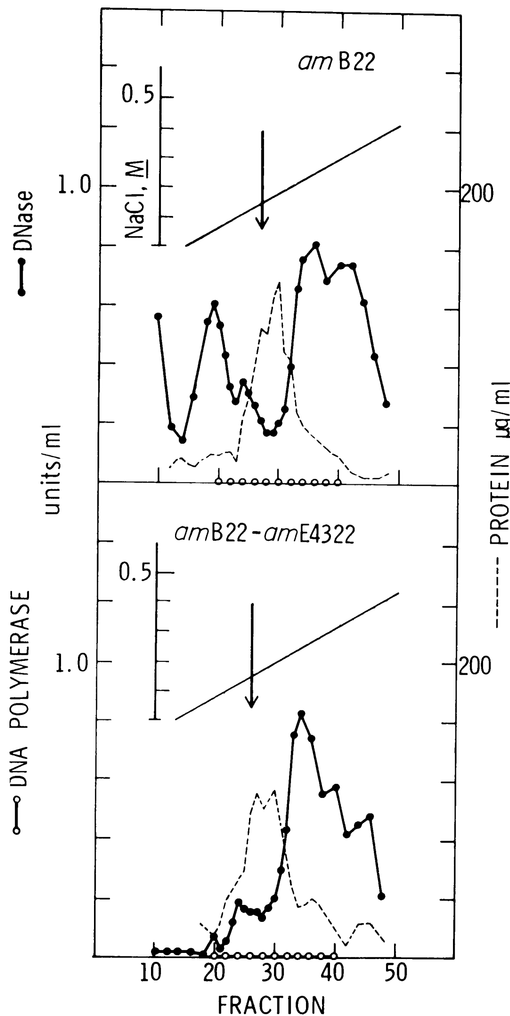

FIG. 4. *DEAE-cellulose chromatography of DNA polymerase and deoxyribonuclease activities in extracts of E. coli W3110 pol A_1^+ str^r cells infected with amB22 (top) and amB22-am E4322 (bottom). The following amounts of protein were applied to the columns in a total sample volume of 8.0 ml in each case: amB22, 6.4 mg; amB22-amE4322, 7.2 mg. Arrow indicates the peak of G6PD activity.*

*am*E4335 signal during translation of the gene 43 messenger. This implies counterclockwise translation for gene 43, although the effects of *am* mutations which map to the counterclockwise side of *am*B22 should also be tested. Unfortunately, the gene 43 *am* mutants which are available do not seem to produce viable combinations for performing such a test. However, we have observed that a suppressible missense mutation which maps to the counterclockwise side of *am*B22 neither abolished nor altered the *am*B22-induced nuclease activity in the su^- host (*unpublished data*).

TABLE 3. *Effect of pCMB on the DNA polymerase activities recovered in the experiments of Fig. 3 and 5*[a]

Expt	Phage mutant	Total DNA polymerase units[b] recovered	
		−pCMP	+pCMB
Fig. 3A	Uninfected cells	10.71 (0.338)[c]	12.06 (0.381)
B	*am*B22	11.12 (0.411)	9.43 (0.350)
C	*am*E4322	6.84 (0.280)	8.78 (0.361)
D	*am*B22-*am*E4322	12.97 (0.421)	14.07 (0.456)
Fig. 5A	*am*E4335	14.08 (0.567)	15.84 (0.633)
B	*am*E4335-*am*E4309	14.87 (0.511)	14.11 (0.486)
C	*am*E4335-*am*E4322	16.27 (0.588)	11.20 (0.406)
D	*am*E4309	10.66 (0.444)	10.42 (0.434)

[a] Abbreviations: pCMB, *p*-chloromercuribenzoate; DNA, deoxyribonucleic acid.
[b] See Materials and Methods.
[c] Values in parentheses represent total DNA polymerase activity recovered per milligram of protein chromatographed.

The B22 and C125 sites are located approximately 80% of the total gene length from the clockwise end of gene 43 on the circular T4 map. Furthermore, these two *am* sites are separated by a recombination frequency of about 0.006 to 0.01%. So, it seems that the residual nuclease activity produced by gene 43 *am* mutants in su^- cells is dependent upon a narrow range of protein fragment sizes. Nossal and Hershfield (26) compared labeled, tryptic peptides of highly purified *am*B22-induced exonuclease and wild-type DNA polymerase. They found that the *am*B22-induced exonuclease consisted of a fragment of the wild-type T4 DNA polymerase molecule with a molecular weight of 80,000 to 90,000. The molecular weight of the wild-type T4 DNA polymerase is about 110,000 (15). These results, therefore, also suggest a counterclockwise reading of gene 43 although other interpretations are possible (26).

Studies by Terzaghi et al. (32) with the lysozyme gene of T4 suggest that transcription and translation are codirectional in vivo. Gene 43 does not seem to be an exception to this notion. The studies of Jayaraman and Goldberg (18) and

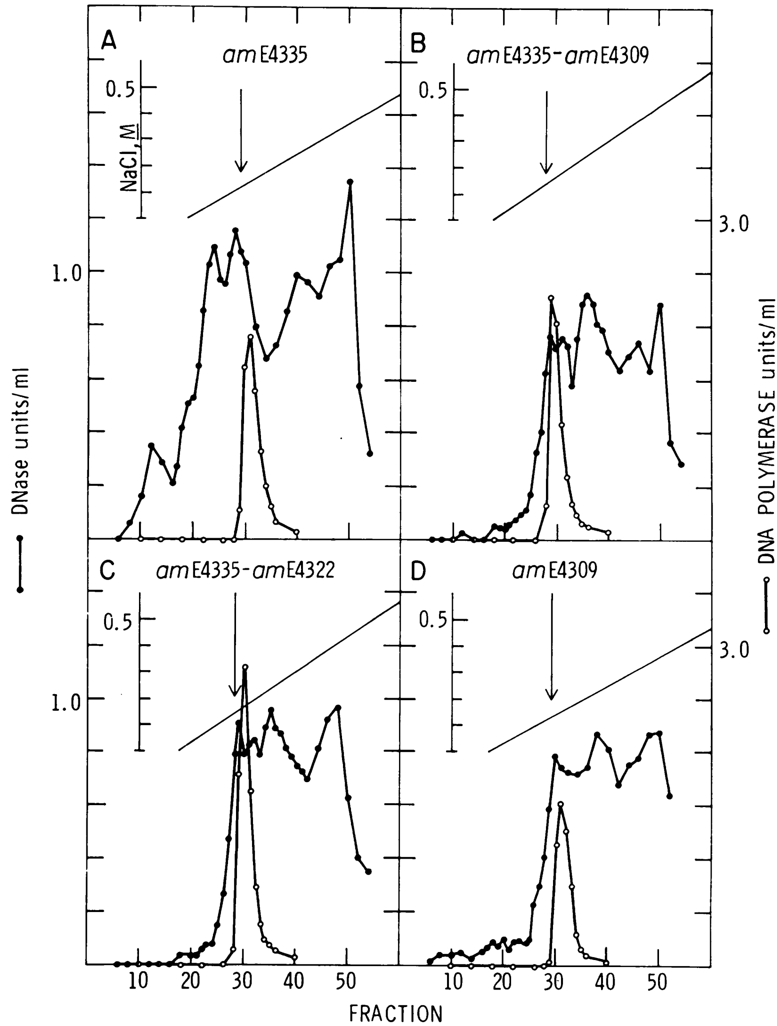

FIG. 5. *DEAE-cellulose chromatography of DNA polymerase and deoxyribonuclease activities in extracts of E. coli W3110 pol A_1^+ str^r cells infected with am mutants of T4 gene 43. Nine milliliters of crude extract containing the following amounts of protein was applied: amE4335, 25 mg; amE4335-amE4309, 29 mg; amE4335-amE4322, 27.6 mg; amE4209, 24 mg. Arrow indicates the peak of G6PD activity.*

Guha et al. (17) suggest that transcription of gene 43 is counterclockwise, and, on the basis of the results reported here and the findings of Nossal and Hershfield, it is reasonable to conclude that the translation for gene 43 is also counterclockwise.

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