Suppression of Amber Mutations of Bacteriophage T4 Gene 43 (DNA Polymerase) by Translational Ambiguity

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The growth properties of twelve different amber (*am*) mutants of bacteriophage T4 gene 43 (DNA polymerase) were examined by using nonpermissive (*su*⁻) as well as permissive (*su*⁺) *Escherichia coli* hosts. It was found that most of these mutants were measurably suppressed in *su*⁻ hosts by translational ambiguity (misreading of codons during protein synthesis). The ability of these mutants to grow in response to this form of weak suppression probably means that the T4 gene 43 DNA polymerase can be effective in supporting productive DNA replication when it is supplied in small amounts. By similar criteria, studies with other phage mutants suggested that the products of T4 genes 62 (uncharacterized), 44 (uncharacterized), 42 (dCMP-hydroxymethylase), and 56 (dCTPase) are also effective in small amounts. Some T4 gene products, such as the product of gene 41 (uncharacterized), seem to be partially dispensable for phage growth since *am* mutants of such genes do propagate, although weakly, in streptomycin-resistant *su*⁻ hosts which appear to have lost the capacity to suppress *am* mutations by ambiguity.

It has been demonstrated for several microorganisms that DNA replication initiates at a specific origin on the DNA genome and proceeds sequentially away from the origin (6, 10, 36, 50, 57, 60, 62). This sequential nature of DNA replication suggests that some of the enzymes and structural proteins which are involved in events occurring at the replication fork become associated with the fork when (or shortly after) replication is initiated, and remain associated with the fork until one, or perhaps several, rounds of replication are completed. Such a replication process would be economical in that it would require only a certain minimal number of molecules of any particular replication enzyme to catalyze several rounds of chromosome duplication.

It is likely that one of the enzymatic activities which are associated with the replication fork is a DNA polymerase. However, it is not yet clear whether any of the known DNA polymerases of bacteria and bacteriophages represent such an enzyme.

Gene 43 of bacteriophage T4 is the structural gene for the only known T4-induced DNA polymerase (15, 40, 58). This polymerase is required for normal phage growth, but its spe-

cific biological role has not yet been explained. The enzyme may be directly involved in semi-conservative phage DNA replication or may indirectly affect this process by controlling DNA synthesis in DNA repair processes which are essential for phage survival under normal growth conditions. We have attempted to differentiate between these two possible roles of the T4 gene 43 DNA polymerase in studies on partial suppression of amber (*am*) mutations of this gene. A collection of T4 gene 43 *am* mutants were characterized by genetic mapping and by studies on their growth properties in bacterial hosts carrying various known suppressors of the amber codon, UAG. The mutants mapped at 12 sites in gene 43 and exhibited a variety of growth responses to UAG suppressors. In addition, it was found that most of these *am* mutants were measurably suppressed by the translational ambiguity of *Escherichia coli* hosts which are generally presumed to be nonpermissive (*su*⁻) for *am*. Transmission coefficients for *su*⁻ hosts infected with the gene 43 mutants ranged from less than 1% to about 100% of cells infected with wild-type phage, depending on the genetic location of the *am* mutation and the *su*⁻ host used. The partial growth of T4 gene

43 *am* mutants in response to the low efficiency of suppression by ribosomal ambiguity is probably a reflection of the direct "catalytic" role of the gene 43 DNA polymerase in DNA replication. It appears that small amounts of the gene 43 polymerase are sufficient to support productive DNA replication in T4-infected cells.

Several T4 genes besides gene 43 are essential for phage DNA synthesis *in vivo* (21, 63). We found that *am* mutants of some of these genes, but not of others, were measurably suppressed by low levels of ribosomal ambiguity. The results support the notion that for each of these genes there is a minimal amount of product which is required to activate *in vivo* DNA replication and that the required minimal amount of gene product is different for different genes. (Portions of this work were submitted by P. V. O'Donnell in partial fulfillment of the requirements for a Ph.D. at the Sloan-Kettering Division, Cornell University Graduate School of Medical Sciences, New York, N.Y., 1973.

MATERIALS AND METHODS

Phage strains. All the T4 phage strains used were originally obtained from R. S. Edgar and W. B. Wood and are derivatives of T4D. They are listed in Tables 1 and 2. The gene 43 *am* mutants were obtained via J. F. Speyer, the gene 42 mutants via J. S. Wiberg, and *am*H18 and *am*HL618 (both in gene 32) via B. M. Alberts. Double-*am* mutants were constructed by genetic crosses, and their composition was verified by (i) their inability to yield wild-type recombinants in crosses with the parental single *am* mutants and by (ii) crosses with wild-type phage which yielded the original single-*am* mutants. The genetic purification of *am* mutants was carried out as follows. Double-triple- or multiple-*am* mutants were first constructed, and then these were crossed to wild-type phage, and the single-*am* mutant recombinants were isolated.

Bacterial strains. The *E. coli* strains which were

used in these studies are described in Table 3. These strains were generous gifts from J. Cairns, C. Davern, R. Edgar, J. Eigner, A. Garen, A. Hershey, M. Oishi, J. Speyer, and N. Zinder. Both P3478 and the W3110 *thy*⁻ strain from which P3478 originated (14) were obtained from J. Cairns. The isolates we used exhibited the same biochemical and biological properties which were described for the original strains by DeLucia and Cairns (14), except that our P3478 did not require thymine (or thymidine) for growth. It is not known how this reversion from *thy*⁻ had been selected. The Bs-1 strain we used was a thymine-requiring mutant which was derived by C. Davern from the original Bs-1. The B3, ER22, S/6, Bs-1, and BB strains are derivatives of *E. coli* B. All the other strains are derivatives of *E. coli* K-12. The designation *su*⁻ is used to denote a lack of suppression by the known suppressors of UAG (amber) and UAA (ochre)

TABLE 1. T4 *am* mutants in gene 43

Site ^a	Mutants at site
E4302	<i>am</i> E4302, <i>am</i> E4303, <i>am</i> E4310, <i>am</i> E4311, <i>am</i> E4312, <i>am</i> E4319, <i>am</i> E4325, <i>am</i> E4329, <i>am</i> E4333, <i>am</i> E4340, <i>am</i> E4341
B22	<i>am</i> B22, <i>am</i> NO20, <i>am</i> NO36, <i>am</i> BU21, <i>am</i> E4323
C125 ^b	<i>am</i> C125, <i>am</i> E4315, <i>am</i> E4335
E4317	<i>am</i> E4317, <i>am</i> E4328, <i>am</i> E4330, <i>am</i> E4337
BU23 ^c	<i>am</i> E4301, <i>am</i> E4305, <i>am</i> E4307, <i>am</i> E4316
E4304	<i>am</i> E4304, <i>am</i> E4339, N101, E4313
E192	<i>am</i> E192, <i>am</i> E4321, <i>am</i> E4326
E4309	<i>am</i> E4309, <i>am</i> E4318, <i>am</i> E4320, <i>am</i> E4331
E4322	<i>am</i> E4322, <i>am</i> NO19
E4314 ^b	<i>am</i> E4314, <i>am</i> E4324
E4332 ^b	<i>am</i> E4332, <i>am</i> E4334
E4306 ^b	<i>am</i> E4306, <i>am</i> E4327, <i>am</i> E4336, <i>am</i> E4338

^a After E. F. Allen, I. Albrecht, and J. Drake (2).

^b See J. Drake (18) for mapping inconsistencies involving these sites.

^c The mutant *am*BU23 is missing from our collection.

TABLE 2. Mutants of T4 DNA metabolism genes

Gene ^a	Product	Amber mutants used ^b	Relevant reference
1	Deoxyribonucleoside monophosphate kinase	<i>am</i> B24	19
32	32-Protein	<i>am</i> A453, <i>am</i> H18, <i>am</i> HL618	1
41	Uncharacterized	<i>am</i> N81	44
42	dCMP hydroxymethylase	<i>am</i> N122, <i>am</i> N55, <i>am</i> C87	16, 61
44	Uncharacterized	<i>am</i> N82, <i>am</i> E4408 ^c , <i>am</i> NG2057	5
45	Uncharacterized	<i>am</i> E10	30
56	dCTPase, dUTPase, dCDPase, dUDPase	<i>am</i> E51, <i>am</i> E114	39, 47
62	Uncharacterized	<i>am</i> E1140, <i>am</i> NG424	5

^a Nomenclature used by W. B. Wood et al. (63).

^b Recombination and complementation tests showed that all the mutants used were different.

^c *Am*E4408 is the same as *am*E4308, a gene 44 mutant which was found in the collection of gene 43 mutants (see reference 2).

TABLE 3. *Escherichia coli* strains

Strain designation	Other designations	Genotype or properties ^a	Relevant reference
<i>su</i> ⁻	S26, K38	<i>P</i> ⁻ <i>su</i> ⁻ (λ)	23, 29
<i>su</i> ⁺ 1 (serine)	S26R1e, K37	<i>P</i> ⁻ <i>su</i> ⁺ 1 (λ)	23, 29
<i>su</i> ⁺ 2 (glutamine)	S26R1d, K146	<i>P</i> ⁻ <i>su</i> ⁺ 2 (λ)	23, 29
<i>su</i> ⁺ 3 (tyrosine)	H12R8a, K110	<i>P</i> ⁻ <i>su</i> ⁺ 3 (λ)	23, 29
<i>su</i> ⁺ 4 (tyrosine)	H12R7a	<i>P</i> ⁻ <i>su</i> ⁺ 4 (λ)	23
<i>su</i> ⁺ 5 (lysine)	U11R1D	<i>P</i> ⁻ <i>su</i> ⁺ 5 (λ)	23
<i>su</i> ⁺ 6 (leucine)	S26 <i>su</i> ⁺ 6	<i>P</i> ⁻ <i>su</i> ⁺ 6 (λ)	12
<i>su</i> ⁺ B	CA165, K145	<i>i</i> ⁻ <i>lac</i> 2 <i>su</i> ⁺ B (λ)	8
CR63		<i>su</i> ⁺ 1λ ^r	3, 55
B		Standard <i>su</i> ⁻ <i>E. coli</i> B strain	21
B3	B ₃ <i>thy</i> ⁻	<i>su</i> ⁻ <i>thy</i> ⁻	4
ER22		<i>su</i> ⁻ <i>endI</i> ⁻	20
S/6		<i>su</i> ⁻ T6 ^r	17
Bs-1		<i>su</i> ⁻ UV sensitive ^b <i>thy</i> ⁻	27, 37
BB	B Berkeley	<i>su</i> ⁻	35
W3110		Standard <i>su</i> ⁻ <i>E. coli</i> K-12 strain	
P3478	<i>polA</i>	<i>su</i> ⁻ <i>polA</i> 1	14
W3110 <i>thy</i> ⁻	Parent of P3478	<i>su</i> ⁻ <i>thy</i> ⁻	14
12P	<i>polA</i> ⁺ revertant ^c of P3478	<i>su</i> ⁻ <i>polA</i> ⁺	

^a *P*⁻ refers to *am* mutations in the gene for alkaline phosphatase; *su*⁺1, *su*⁺2, *su*⁺3, and *su*⁺6 are suppressors of *am* mutations; *su*⁺4, *su*⁺5 and *su*⁺B are suppressors of ochre mutations which can also suppress *am* mutations; *su*⁻ is the wild-type counterpart of *su*⁺; λ^r and T6^r mean resistance to phages λ and T6, respectively; *thy*⁻ means thymine requiring; *endI*⁻ refers to a defect in endonuclease I activity; *polA*1 is an *am* mutation in the gene for DNA polymerase I.

^b The high sensitivity of Bs-1 to UV irradiation is due to two mutations: *hcr*⁻ (*uvrB*) and *exr*⁻.

^c Isolated on EHA plates in the presence of 0.04% methyl methane sulfonate.

codons. It is not used to describe the effects of ribosomal ambiguity. Thus an *su*⁻ bacterial strain may or may not possess the capacity to suppress point mutations via errors in protein synthesis.

Media and growth conditions. EHA bottom agar and EHA top agar (54) were used as media for plates. Liquid media were the modified M9 medium previously described (42), and a broth (NB) medium which contained 0.8% nutrient broth (Difco) and 0.5% sodium chloride in aqueous solution and which was adjusted to pH 7.4 with sodium hydroxide prior to sterilization. The solid medium which was used for preparing bacterial stabs and slants consisted of 1.2% agar (Difco) in NB medium. When M9 medium was used for thymine-requiring bacteria, it was supplemented with 5 μg of thymidine per ml.

Main stocks of all bacterial strains were stored at room temperature in paraffin-sealed NB agar stabs and remained viable for several years. These stabs were used to prepare stocks which were maintained at room temperature on NB agar slants in tightly capped screw-cap tubes and which could be used for inoculations over a period of several months. The slant cultures were used to inoculate NB medium which, when incubated for about 15 h at 30 C without aeration, yielded cultures at about 10⁸ cells per ml. These cultures were stored in a refrigerator (4–8 C) and then were used as stock cultures for a period of 10 to 14 days. Overnight cultures were prepared by inoculating 2 ml of growth medium with about 0.01 ml of refrigerated stock culture and incubating the inoculated media at 30 C without aeration for 15 to 17 h.

Daily working cultures were prepared by diluting overnight cultures 50-fold in fresh growth medium and aerating the inoculated media at 37 C until the desired cell density was obtained. Overnight cultures were prepared in the same media which were to be used for the daily working cultures. With most of the *E. coli* strains used, the cell density did not exceed 2 × 10⁸ per ml when growth was in NB medium without aeration. With aeration, the highest cell concentrations which could be obtained with NB medium was near 2 × 10⁹ cells per ml. In M9 medium, cell concentrations in the order of 3 × 10⁹ could be obtained whether or not the cultures were aerated. Cell concentrations were determined by microscope count with a Petroff-Hausser bacteria counter (A. H. Thomas, Co., Philadelphia, Pa.).

The media and growth conditions which were used in experiments on the preparation of crude extracts, DEAE-cellulose chromatography, and DNA polymerase assays were as previously described (42).

Streptomycin-resistant (*str*^r) derivatives of some of the bacterial strains were isolated on EHA plates containing 200 μg of streptomycin per ml, by using the following procedure. The bacteria were grown in NB medium to a density of about 10⁸ cells per ml, the cells were harvested by centrifugation, and the cell pellet was suspended in broth to yield a cell concentration of about 5 × 10¹⁰/ml. About 10¹⁰ cells were spread on each of several streptomycin-containing plates, and the plates were incubated at 37 C. *str*^r colonies were visible after about 15 h of incubation and were generally picked by 20 to 24 h after incubation. The

picked colonies were then purified by being streaked on streptomycin-EHA plates, and stocks were subsequently prepared from purified colonies.

Phage crosses. Qualitative recombination tests, in which the number of *am* sites in T4 gene 43 was determined, were performed as follows. One drop (about 0.05 ml containing 5×10^6 phage particles) of each of any two phage mutants being tested against each other were mixed in wells of a model 96U-CS-Clear Dispo Tray (Linbro Chemical Co., Inc., New Haven, Conn. 06511), and two drops (about 0.1 ml) of *E. coli* CR63 cells (about 10^8 cells) were added to each of the phage mixtures. Adsorption of the phage to the cells was permitted to occur at room temperature for 15 min, after which four drops of dense *E. coli* S/6 *str*^r (3×10^8 cells/ml) were added to the infection mixtures. Warm (45–50 C) EHA top agar was then dispensed into the wells, and the trays were covered with Saran Wrap. The agar was permitted to gel at room temperature before the trays were transferred to a 30 C incubator for overnight incubation. Recombination was indicated by the appearance of more plaques in wells containing the mixture of mutants than in the control wells which utilized only one or the other phage strain used in the cross.

Qualitative spot tests. The ability of a T4 phage mutant to grow on a certain bacterial host could be readily tested by spotting approximately 5 μ liters of phage solution (at 5×10^7 phage/ml) onto an EHA plate which had received approximately 2×10^8 cells with the EHA top agar. Up to 33 phage solutions could be spotted per plate when grids drawn on index cards were placed under the plates to guide the spotting.

Transmission coefficients. Measurements of transmission coefficient (the fraction of infective centers which yields at least one viable phage particle) were carried out in M9 medium as follows. Cells were grown at 37 C to a density of about 3×10^8 cells per ml, and then they were diluted to about 10^8 cells per ml and aerated at 30 C for 60 min. These cultures were then adjusted to 10^8 cells per ml with fresh medium, and 0.5-ml fractions were dispensed into tubes which had been prewarmed to 30 C. Phage was then added to the cells to yield a multiplicity of infection of 10 and, after 10 min of adsorption, T4 antiserum was added (final titer $k = 2/\text{min}$) to remove unadsorbed phage. At 20 min after infection, the cultures were diluted in M9 medium and plated for infective center titer on *E. coli* CR63. In experiments where burst size (average phage yield per infective center) measurements were also taken, a 10^2 -fold dilution of the infection mixture which was taken at 20 min after infection was kept at 30 C for 2 h, lysed with CHCl_3 , and assayed for phage yield on *E. coli* CR63. The infective center titer of the wild-type control was used in the computation of all burst sizes. In experiments where the effects of streptomycin on suppression were measured, the drug was added to cells at 20 min before infection to yield a final concentration of 3 $\mu\text{g}/\text{ml}$ and was present at this concentration in all the liquid media used in the experiment. Platings for infective centers and for phage yields were performed on EHA plates devoid of

streptomycin and by using indicator cells which had not been treated with the drug.

RESULTS

The T4 gene 43 *am* map. About 50 *am* mutants of T4 gene 43 have been isolated by R. S. Edgar and his collaborators (personal communications). One collection of these mutants was studied by Drake and his collaborators (2), and we studied a presumably identical collection which was independently obtained from Edgar. Some mapping inconsistencies were observed between the two collections, and these were recently summarized by Drake (18). A listing of the mutants in the collection we used appears in Table 1. These mutants were assigned to gene 43 sites on the basis of qualitative recombination tests. Studies which utilized two- and three-factor crosses (24; unpublished results) generally confirmed the order of gene 43 *am* sites which was determined by Allen et al. (2). In Table 1, the descending order of sites corresponds to the clockwise order of these sites on the circular T4 genetic map. Our studies also confirmed that two types of mutations have been isolated for the E4304 site: (i) amber (*am*E4304 and *am*E4339) and (ii) a non-amber (N101 and E4313) mutation which has been suspected to be a leaky missense mutation (35, 58). We have examined E4313 and obtained results which suggest that it is a UGA nonsense mutant rather than a missense mutant. The evidence was as follows. (i) E4313 grew at higher efficiency in bacterial strains carrying UGA suppressor mutations than on bacterial strains lacking such suppressors. (ii) 5-Bromodeoxyuridine mutagenesis of E4313 resulted in the isolation of mutant derivatives which had the properties of ochre (UAA) mutants. (iii) 2-Aminopurine mutagenesis of these apparent ochre mutants resulted in the isolation of both, derivatives which had the phenotype of E4313 and derivatives which had the phenotype of *am*E4339. (iv) We detected a small amount of recombination (about 0.001% wild type) between E4313 and *am*E4339 and between E4313 and the apparent *am* derivatives of this mutant. However, no recombination was detected (less than $10^{-4}\%$) between E4313 and its apparent ochre derivatives, between the apparent ochre mutants and *am*E4339, or between *am*E4339 and the apparent *am* mutants which were derived from E4313 via ochre. These observations and the fact that both E4313 and *am*E4339 were originally obtained after 2-aminopurine mutagenesis of wild-type phage (R. Edgar,

personal communication) suggest that the wild-type codon for the E4304 site is UGG (tryptophan).

Suppression of T4 gene 43 mutants by amber and ochre suppressors. In preliminary studies, all the gene 43 mutants in our collection were tested for growth properties on several different *su*⁺ *E. coli* hosts by using two temperatures, 30 and 42 C. In several instances, mutants which mapped at the same site gave different growth responses. These inconsistencies among presumable identical mutants were found to be due to secondary mutations in the genomes of some of the phage stocks. These secondary mutations were probably the by-product of the mutagenic treatments which were originally used to produce the gene 43 *am* mutations. The *am* isolates which we used in the following experiment had all been backcrossed to remove the effects of mutations other than the *am* in gene 43.

Qualitative spot tests were used to examine the growth properties of various gene 43 *am* mutants on various *E. coli su*⁺ hosts. Some results are described in Table 4. The following observations and explanations may be made.

(i) Four major types of responses were obtained: (a) normal growth, (b) weak growth, (c) temperature-sensitive growth, and (d) no growth, depending on the bacterial host and phage mutant used. In another experiment (results not shown) qualitatively similar results were obtained at 25 and 30 C. That is, no cold-sensitive (growth at 42 C, but not at 25 or 30 C) responses were obtained.

(ii) The mutant *amE4311* (E4302 site) grew

abnormally in response to all of the *su*⁺ host mutations which we have examined. This probably means that the E4302 genetic site corresponds to an amino acid site in the DNA polymerase protein which is very critical for activity of the enzyme. The E4302 mutants, as well as all the mutants carrying the prefix E, were isolated after 2-aminopurine mutagenesis (Edgar, personal communication). This mutagen presumably generates *am* (UAG codon) from wild-type DNA sequences which code for glutamine CAG codon) and tryptophan (UGG codon) (9, 22). It is likely that the E4302, E4306, E4314, and E4317 *am* site mutations arose from tryptophan in the wild-type (2) since glutamine substitution (*su*⁺2) did not result in normal growth of any of these mutants.

(iii) The mutant *amE4301* fails to grow, or grows very poorly, in response to suppressors which insert tyrosine for UAG. This probably means that tyrosine is not acceptable for enzyme activity when it is inserted at a location in the polymerase corresponding to the E4301 genetic site. It is also possible that the efficiencies of suppression by *su*⁺3 and *su*⁺4 are very low for this particular site due to effects of other base sequences in the messenger (49). A similar explanation may be used for the apparent weak suppression of E4302 site mutations by *su*⁺2.

(iv) Some of our results on the suppression patterns of the gene 43 mutants differ from the results of Allen et al. (2). Some of the differences may be accounted for by the fact that we used, on the most part, a different family of *su*⁺ host strains which may have different efficiencies of *am* suppression. Other differences

TABLE 4. Suppression of T4 gene 43 *am* mutations by amber and ochre suppressors

Site	Representative mutant	Growth response ^a on							
		<i>su</i> ⁻	<i>su</i> ⁺ 1 (<i>ser</i>)	<i>su</i> ⁺ 2 (<i>gln</i>)	<i>su</i> ⁺ 3 (<i>tyr</i>)	<i>su</i> ⁺ 4 (<i>tyr</i>)	<i>su</i> ⁺ 5 (<i>lys</i>)	<i>su</i> ⁺ 6 (<i>leu</i>)	<i>su</i> ⁺ B
E4302	<i>amE4311</i>	—	+(-)	vw(-)	+(vw)	+(-)	-(-)	-(-)	vw(-)
B22	<i>amB22</i>	—	+(+)	+(+)	+(+)	+(w)	+(w)	+(+)	+(+)
C125	<i>amE4335</i>	vw	+(+)	+(+)	+(-)	+(-)	+(w)	w(-)	+(+)
E4317	<i>amE4317</i>	—	+(w)	+(w)	+(+)	+(+)	+(-)	+(w)	+(-)
BU23	<i>amE4301</i>	—	+(+)	+(+)	-(-)	-(-)	+(+)	+(w)	+(+)
E192	<i>amE4321</i>	w	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)
E4304	<i>amE4339</i>	—	+(+)	+(+)	+(+)	+(+)	+(-)	+(w)	+(+)
	E4313	w	w(w)	w(w)	w(w)	w(w)	w(w)	w(w)	+(+)
E4309	<i>amE4309</i>	—	+(+)	+(+)	+(w)	+(w)	+(-)	w(-)	+(w)
E4322	<i>amE4322</i>	vw	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)
E4314	<i>amE4314</i>	—	+(w)	+(w)	+(+)	+(w)	-(-)	+(-)	+(w)
E4306	<i>amE4327</i>	—	+(-)	+(-)	+(+)	+(w)	-(-)	+(-)	+(-)
E4332	<i>amE4332</i>	—	+(+)	+(+)	+(+)	+(+)	+(w)	+(+)	+(+)

^a Growth response was determined relative to wild-type phage: +, growth equal to that obtained with wild-type; w, growth weaker than wild-type; vw, growth weaker than "w" responses; and —, no growth. The notations in parentheses are growth responses at 42 C; notations outside parentheses are responses at 30 C.

may be due to secondary mutations in the phage stocks which were used by Allen et al. Most significant, perhaps, are the differences in results on suppression with *su*⁺B, an ochre (UAA) suppressor. Presumably, the same host strain (*E. coli* CA165) was used in the two studies. In contrast to our results, Allen et al. obtained generally poor or no growth of T4 gene 43 *am* mutants in response to *su*⁺B. We have observed that *E. coli* CA165 tends to lose its suppression capability during prolonged storage or during serial subculturing. This may not be too surprising since ochre-suppressing bacterial strains do not grow as efficiently as their *su*⁻ counterparts (43, 46), and selection for *su*⁻ may occur during growth. Our results suggest that *su*⁺B inserts the same amino acid which is inserted by *su*⁺2, namely glutamine.

(v) Most of the gene 43 *am* mutants were suppressible by the other ochre (UAA) suppressors, *su*⁺4 and *su*⁺5, which are known to suppress UAG at low efficiency.

Suppression of T4 gene 43 *am* mutants in *su*⁻ hosts. In the experiments for Table 4, it was observed that some *am* mutants of gene 43 gave weak growth responses when they were tested on *E. coli* K38, an *E. coli* K-12 strain which is not known to suppress *am* mutations (23, 29). It was suspected that the weak growth was due to ribosomal ambiguity: the ribosome-mediated occasional translation of UAG non-

sense to sense (25). A number of other presumed *su*⁻ *E. coli* strains were tested for their ability to support the growth of T4 gene 43 *am* mutants. Derivatives of both *E. coli* K-12 and *E. coli* B were used (Table 5). Partial growth on one *su*⁻ host or another was observed with most of the *am* mutants tested and with the UGA mutant E4313. These results suggest that the ability to partially suppress T4 gene 43 *am* mutations is a property common to most *su*⁻ *E. coli* strains.

In the experiments for Table 5 and in other studies, several types of observations indicated that the growth of these *am* mutants on *su*⁻ hosts was due to errors in the fidelity of translation of the gene 43 messenger rather than, for example, to substitution of a host DNA polymerase activity for the missing gene 43 polymerase.

(i) Most of the mutants were leaky on *E. coli* P3478, a host strain which lacks DNA polymerase I (14). Growth was not improved on a *polA*⁺ derivative of this host.

(ii) It is known that host mutations to streptomycin-resistance result in ribosomal protein changes which interfere with the expression of ribosomal ambiguity (24, 45). Table 5 shows that the *str*^r derivatives of the *su*⁻ bacterial strains which we used had largely lost the ability to support the weak growth of the phage mutants. In other experiments we observed that *am* mutants of several T4 genes, including genes

TABLE 5. Transmission of T4 gene 43 *am* mutants in *su*⁻ *E. coli* hosts

Mutants	Transmission coefficient (%) ^a for phage-infected <i>E. coli</i>												
	B	B3	Bs-1	ER22	BB	S/6	S/6 <i>str</i> ^r ^b	W3110	P3478	12P	W3110 <i>thy</i> ⁻	K38	K38 <i>str</i> ^r ^b
<i>am</i> E4311	0.1	0.1	<0.03	<0.03	0.2	0.2	0.02	0.2	<0.03	0.05	0.1	0.03	0.1
<i>am</i> B22	18	2.5	29	13	18	8	<0.1	10	7	8	20	8	0.04
<i>am</i> E4335	56	83	80	49	30	50	<0.1	43	43	70	96	68	0.3
<i>am</i> E4317	44	74	91	86	30	34	0.2	45	43	45	60	46	0.1
<i>am</i> E4301	19	6	46	17	16	9	0.5	26	17	14	29	10	0.1
<i>am</i> E4321	88	92	97	68	82	60	0.5	62	46	64	60	60	0.2
<i>am</i> E4339	0.6	0.2	0.9	10	0.4	0.1	0.1	5	1	1.5	15	1	0.2
E4313	70	100	74	54	100	51	<0.1	55	60	73	71	97	<0.04
<i>am</i> E4309	28	28	40	65	19	6	0.2	47	29	30	56	35	0.2
<i>am</i> E4322	26	16	51	21	11	10	0.2	30	16	24	36	24	0.2
<i>am</i> E4314	5	1	31	26	0.3	2.5	<0.1	9	3	4	9	0.5	0.1
<i>am</i> E4327	0.4	<0.1	2	0.03	0.1	0.3	0.05	0.4	0.05	0.1	1	0.05	0.1
<i>am</i> E4332	54	54	68	35	47	18	<0.1	43	21	55	44	30	0.3
<i>am</i> B22-E4322	<0.02	<0.1	<0.03	<0.03	<0.05	<0.02	0.03	<0.02	<0.03	<0.05	<0.04	<0.03	<0.04
<i>am</i> E4335-E4322	0.02	<0.1	<0.03	<0.03	<0.05	<0.02	0.05	<0.02	<0.03	<0.05	<0.08	<0.03	0.1
<i>am</i> E4301-E4321	<0.02	<0.1	<0.03	<0.03	<0.05	<0.02	<0.03	<0.02	<0.03	<0.05	<0.04	<0.03	<0.04
T4D ⁺⁺	100	100	100	100	100	100	100	100	100	100	100	100	100

^a Transmission coefficients were determined relative to T4 wild-type (T4D⁺⁺)-infected bacteria.

^b Very similar results were obtained with *str*^r derivatives of Bs-1, BB, P3478, W3110, and a *pol*⁺ revertant of P3478*str*^r.

which control phage assembly, were also leaky on streptomycin-sensitive *su*⁻ *E. coli* hosts, but not on *str*^r derivatives of these hosts. It is unlikely that host enzymes and other proteins can substitute for all of these phage functions.

(iii) Different *am* mutants of gene 43 exhibited different levels of growth when compared in the same *su*⁻ host. This probably means that the efficiency of growth is dependent on the quality, as well as the quantity, of the gene 43 product which is synthesized in response to ambiguity.

(iv) Double-*am* mutants of T4 gene 43 failed to propagate in *su*⁻ hosts which supported the partial growth of the corresponding single-*am* mutants. Such results may be expected if misreading of nonsense codons occurred at low frequency. The probability of misreading two UAG codons in one sequence of translation is probably very low indeed.

(v) The *am* mutant of the E4302 site (*amE4311*) yielded very weak growth under the *su*⁻ conditions described. This result is consistent with the observations that *amE4311* yielded abnormal growth with all suppressors (Table 4). The low efficiency of translational ambiguity and the limitations in acceptable amino acid substitution for *amE4311* probably both contribute to the non-leaky response of this mutant in *su*⁻ hosts.

(vi) Burst-size measurements were also performed in the experiments for Table 5, and, as expected, measureable phage yields were obtained with most of the mutants. Representative results are shown in Table 6. Included in Table 6 are the results on the effects of sublethal doses of streptomycin. In *E. coli* S/6 the presence of 3 μ g of streptomycin per ml enhanced the growth of single-*am*, but not double-*am*, mutants of gene 43. In other experiments (not shown) visible, but tiny, plaques could be obtained at high frequency in platings of some of the gene 43 *am* mutants (e.g., *amE4335*) on some of the *su*⁻ hosts (e.g., W3110 *thy*⁻). Also, the leakiness of most of the gene 43 mutants in *su*⁻ hosts could be easily demonstrated in qualitative spot tests such as those described for K38(λ) in Table 4.

It should be pointed out that the phage mutants seemed to grow at different efficiencies on different *E. coli* B derivatives and different *E. coli* K-12 derivatives. These variations among derivatives of the same bacterial strain probably reflect both the effects of the mutagenic treatments which were originally used to produce the derivatives and the different methods which were used in different laboratories to propagate and store these strains. The set of

TABLE 6. Phage production in *su*⁻ *E. coli* infected with T4 gene 43 *am* mutants

Phage	Burst size ^a on <i>E. coli</i>		
	W3110	S/6	S/6 + streptomycin
<i>amE4311</i>	0.03	0.02	4×10^{-3}
<i>amB22</i>	5.5	0.4	29
<i>amE4335</i>	10	8	15
<i>amE4317</i>	38	11	32
<i>amE4301</i>	6.6	0.6	13
<i>amE4321</i>	45	34	68
<i>amE4339</i>	0.7	0.06	10
E4313	16	18	18
<i>amE4309</i>	8.3	1.1	18
<i>amE4322</i>	6.2	1.5	15
<i>amE4314</i>	1	0.1	5
<i>amE4327</i>	0.04	0.02	0.03
<i>amE4332</i>	26	4	52
<i>amB22-E4322</i>	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
<i>amE4335-E4322</i>	10^{-3}	$<10^{-3}$	$<10^{-3}$
<i>amE4301-E4321</i>	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$
T4D ⁺⁺	94	246	80

^a Average phage yield per infective center.

gene 43 mutants which is described here provides a simple means of determining how permissive any presumed *su*⁻ *E. coli* host is.

Properties of *str*^r derivatives of *E. coli*. Some bias entered our methods of isolation of streptomycin-resistant derivatives of *su*⁻ *E. coli* strains. Spontaneous *str*^r mutants are known to occur very infrequently in cultures of *E. coli* (7). We experienced difficulties in isolating such derivatives from certain bacterial strains. No *str*^r mutants could be derived from *E. coli* B3*thy*⁻ and *E. coli* W3110*thy*⁻ after plating about 5×10^{10} cells of each on EHA agar plates containing 200 μ g of streptomycin per ml. In the case of *E. coli* ER22, some *str*^r colonies were observed, but these were very slow-growing and could not be kept viable under our experimental conditions. The isolates which we did obtain from *E. coli* S/6, BB, Bs-1*thy*⁻, K38(λ), and P3478 also grew more slowly than the streptomycin-sensitive parental strains, but the growth rates were sufficiently high to permit their use in experiments. All such *str*^r strains exhibited a decreased ability to support the growth of the leaky T4 *am* gene 43 mutants. Occasionally, *str*^r derivatives were found which had similar growth rates to the drug-sensitive parents, and these seemed to have an unaltered efficiency of suppression by ambiguity. Although these derivatives may be defective in uptake of streptomycin rather than in ribosomal structure, it is not necessary to assume that all the *str*^r mutations which result in alteration of

ribosomal protein structure also result in altered ribosomal ambiguity (56). We also observed that, during storage at room temperature in nutrient stabs and slants, some of the *str^r* mutants which we isolated gradually regained some of the ability to suppress by ambiguity without losing the *str^r* character. The reappearance of ambiguity was accompanied by an improvement in growth rate. These properties of the bacterial strains were not investigated further.

DNA synthetic capacity of *su⁻* *E. coli* infected with T4 gene 43 *am* mutants. The results in Fig. 1 show the differences in *in vivo* DNA synthetic activities among different T4 *am* gene 43 mutants infecting the *su⁻* host, *E.*

coli P3478. These results are consistent with the results which were obtained in studies which measured the effects of ambiguity on phage production (Tables 5 and 6). *AmE4335* and the UGA mutant *E4313* were among the leakiest of the gene 43 mutants tested. Double-*am* mutants did not seem to respond to the effect of translational ambiguity, and results obtained with P3478 *str^r* infected with single-*am* mutants (not shown) demonstrated that mutants which exhibited DNA synthetic activity in P3478 failed to show such activity in the *str^r* derivative of this host.

To get some estimate of the levels of T4 DNA polymerase activity which were involved in the different efficiencies of growth of the various

FIG. 1. Thymidine incorporation in *E. coli* P3478 infected with T4 gene 43 *am* mutants. The experiments were performed in M9 medium at 30 C. Log-phase cells (10^8 per ml) were infected at a multiplicity of 10 with the phage in question. At 10 min after infection, the infection mixtures were diluted 10-fold in medium containing ^3H -thymidine ($53 \mu\text{Ci}$ of ^3H per ml at a specific activity of $66.5 \mu\text{Ci}$ of ^3H per μg of thymidine). Samples were withdrawn at 10-min intervals after dilution into isotope, and trichloroacetic acid-precipitable radioactivity was determined by using the methods previously described (31).

gene 43 mutants, we assayed for polymerase activity in vitro. *E. coli* P3478 was used as the host since it had a reduced level of in vitro DNA-synthesizing activity due to the *polA* mutation. DNA polymerase activity was determined on pooled fractions from DEAE-cellulose chromatography of crude extracts of infected cells (42). This approach for determining the phage-induced polymerase activity was limited in that DNA polymerase was detectable only with some of the mutants which exhibited high transmission coefficients. The data in Table 7 summarizes some of the observations which were made on in vivo and in vitro activities with infected P3478. The data show that the levels of recoverable DNA polymerase activity did not always reflect the efficiency of phage growth. Several factors may be responsible for the discrepancies, among which are the relative stabilities of the qualitatively different phage-induced DNA polymerases to extraction procedures and to assay conditions. Thus, as may be expected, the amount of enzyme activity which is determined in vitro does not always reflect the catalytic capacity of the enzyme in vivo.

Properties of other T4 DNA metabolism genes. The results which were obtained with T4 gene 43 *am* mutants suggested that small (catalytic) amounts of T4 gene 43 DNA polymerase are sufficient to support productive DNA replication. In addition to its requirement for the gene 43 DNA polymerase, T4 DNA replication requires the products of several of the genes which are expressed during early times after infection. We examined *am* mutants of these genes to see whether they behaved similarly to the gene 43 mutants. The results shown in Table 8 compare the efficiencies of growth of the mutants on two hosts: (i) W3110, which harbors

the ability to suppress by ambiguity and (ii) a *str^r* derivative of the W3110 family which was among the most restrictive *su⁻ str^r* strains we isolated. The types of responses which were obtained may be summarized as follows.

(i) The results obtained with mutants of genes 44 and 62 suggest that the products of these two genes are analogous to the gene 43 product in their essential and catalytic effects in replication.

(ii) None of the three mutants of gene 32 were measurably suppressed in the *su⁻* hosts. These

TABLE 8. Suppression of T4 *am* mutants of DNA metabolism genes by translational ambiguity

<i>am</i> Mutant	Gene affected	Growth* on <i>E. coli</i>	
		W3110	P3478 <i>polA1⁺str^r</i>
B24	1	0.1 (0.02)	0.3 (0.01)
A453	32	0.02 (<0.01)	<0.03 (<0.01)
H18	32	0.1 (0.01)	0.15 (<0.01)
HL618	32	0.03 (0.04)	0.06 (<0.01)
N81	41	10 (37)	6 (2)
N122	42	0.1 (<0.01)	0.03 (<0.01)
N55	42	0.3 (0.02)	0.2 (<0.01)
C87	42	2.4 (0.6)	<0.03 (<0.01)
E4301	43	58 (37)	0.2 (0.1)
N82	44	0.2 (0.1)	<0.03 (0.01)
E4408	44	44 (20)	0.1 (0.2)
NG2057	44	0.1 (<0.01)	0.03 (<0.01)
E10	45	0.5 (0.1)	0.06 (<0.01)
E51	56	0.2 (0.04)	0.06 (<0.01)
E114	56	12.5 (6)	<0.03 (<0.01)
E1140	62	40 (37)	0.06 (0.03)
NG424	62	0.3 (<0.01)	0.05 (<0.01)
Control	T4D ⁺⁺	100 (115)	100 (223)

* Values outside parentheses are transmission coefficients (%) relative to wild-type. Values within parentheses are burst sizes.

TABLE 7. Suppression of T4 gene 43 *am* mutants in *E. coli* P3478

Phage mutant	In vivo					In vitro	
	Trans. coef. (%) ^a	Burst size		Rate of thymidine incorporation		T4 DNA polymerase	
		Phage/cell	% of wild-type ^a	Counts/min	% of wild-type ^b	Specific activity ^c	% of wild-type ^d
<i>amE4335</i>	43	10	4	700	17	0.124	7.6
<i>amE4317</i>	43	21	7	335	8	0.009	0.6
<i>E4313</i>	60	11	4	1,060	25	0.233	14.2
<i>amE4321</i>	46	43	15	470	11	0.003	0.2
<i>amE4309</i>	29	3	1	365	9	0.003	0.2

^a Relative to wild-type (T4D⁺⁺).

^b Based on T4D⁺⁺ rate of 4,200 counts/min obtained in the experiment for Fig. 1.

^c Specific activity = combined polymerase units recovered per milligram of protein chromatographed (42).

^d Based on *amE1140* (gene 62) control which gave a specific activity of 1.63 U/mg of protein. The *amE1140* mutant was chosen as control because it resembles gene 43 *am* mutants in being leaky on the P3478 host.

results are consistent with the explanation of Snustad (51, 52) that the gene 32 product is required in "stoichiometric" amounts. The small amounts of 32-protein which are probably synthesized in response to translational ambiguity in W3110 are probably insufficient to carry out the function which is catalyzed by this protein.

(iii) Although only one mutant of gene 45 (*amE10*) was studied, the results obtained may be meaningful. Gel electrophoretic assays seem to indicate that the gene 45 product is normally synthesized in large amounts relative to other T4 "early" gene products (30). This gene product may also be required in large amounts. The near absence of leaky growth by *amE10* in W3110 may mean that insufficient amounts of gene 45 product are made under these conditions to allow replication to occur.

(iv) Other work suggests that the products of genes 1, 42, and 56 are involved in the synthesis of T4 DNA precursors. It may be expected that high precursor concentrations would be required to satisfy the needs of DNA replication. Thus, it was surprising that mutants in gene 42 (*amC87*) and gene 56 (*amE114*) exhibited some growth in response to translational ambiguity. It is possible that the two mutations, *amC87* and *amE114*, are suppressed at abnormally high efficiency or that the mutations are suppressed at normal efficiency, but that the gene products synthesized harbor abnormally high enzymatic activity. However, other explanations would be considered. For example, dCMP-hydroxymethylase may be involved in base selection (13), and the enzyme which is synthesized by *amC87* in the W3110 host may be particularly efficient in selecting hydroxymethylated precursor for incorporation into DNA. In the case of *amE114*, it is possible that the small amount of dCTPase which is synthesized by this mutant actively selects against the incorporation of dCTP.

(v) The mutant *amN81* grew, although weakly, on both W3110 and on the *str^r su⁻* strain. The growth on the *str^r* host was less efficient than growth on the drug-sensitive host. These results suggest two properties for the gene 41 product: (i) it participates catalytically in replication (*amN81* is sensitive to suppression by ambiguity), and (ii) it is partially dispensable (*amN81* grew in the absence of suppression by ambiguity). It is possible that a host gene product can partially substitute for a missing T4 gene 41 product. Other experiments (unpublished) showed that *am* mutants of T4 genes 33, 39, 46, 47, 52, 58, 59, 60, and 61 are leaky on *str^r* hosts which completely restricted

the growth of *am* mutants of genes 43, 44 and 62. These observations suggest that many T4 gene products are either partially dispensable or can be substituted for by host gene products.

DISCUSSION

The results of this study offer some implications concerning the biological function of the T4 gene 43 DNA polymerase.

All the DNA polymerases which have so far been studied catalyze essentially one type of in vitro DNA synthetic reaction. That is the sequential addition of nucleotides to the 3'-hydroxyl end of a growing DNA chain (26, 33, 34, 38). It is possible that such a synthetic reaction is an essential part of the process of in vivo DNA replication, although it is also possible that DNA replication involves a novel type of DNA synthetic process and that the known DNA polymerases represent examples of both essential and nonessential repair enzymes.

The amount of a DNA polymerase which is found in growing cells may be a reflection of the type of function—repair or replication—which is performed by the polymerase in vivo. Pertinent examples may be found among the three known DNA polymerases of *E. coli*. In extracts of *E. coli* DNA polymerase I (pol I) represents the largest amount of DNA-synthesizing activity (33, 34, 38). DNA polymerases II (pol II) and III (pol III) are apparently synthesized in smaller amounts than pol I in normal cells. Pol I may not be required for DNA replication since the loss of this enzyme by mutation does not seem to affect cell viability under standard growth condition (14). Similarly, pol II can apparently also be eliminated by mutation without loss of cell viability (11, 28). On the other hand, pol III, the product of the *E. coli dnaE* gene (24, 41), is required for normal levels of in vivo DNA synthesis and for normal cell growth (59).

It is possible that pol III specifically catalyzes DNA synthesis at replication growing points (of which there are only few per bacterial cell) and may therefore not be required in large amounts. In contrast, pol I is known to be essential for DNA repair synthesis after irradiation of *E. coli* with UV or treatment of cells with alkylating agents (14). The recovery of cells under these conditions may require the simultaneous repair of several DNA lesions per genome and may therefore require large amounts of pol I.

In T4-infected *E. coli*, the replicating phage DNA pool may contain as many as 60 growing points (60) and may therefore involve the simultaneous functioning of several molecules of a replication polymerase. A reduction in the num-

ber of molecules of replication polymerase would probably lead to a reduction in the number of growing points in the pool without resulting in a complete inhibition of phage DNA replication. Thus, a property of the T4 DNA replication polymerase may be its ability to support productive DNA replication even when it is present at a low specific activity. Our studies show that the T4 gene 43 DNA polymerase exhibits such a property.

The majority of available *am* mutants of T4 gene 43 showed some growth in response to suppression by translational ambiguity—a form of suppression which is known to be of low efficiency (25, 48). The efficiency of phage growth varied with the map location of the *am* mutations under study. This was expected since the suppression of mutations at different locations in a gene represents amino acid substitution at different locations in the protein product of the gene. Such variations in the primary structure of an enzyme may well result in variations of its enzymatic activity. The significant result from our studies is that levels of T4 gene 43 DNA polymerase which are much lower than those which are found in cells infected with normal phage are sufficient to support productive DNA replication. Thus, the T4 gene 43 DNA polymerase resembles the *E. coli* pol III in two respects: (i) it is required for in vivo DNA synthesis and growth and (ii) it is not required in large doses. Also, like *dnaE* (pol III) mutations, temperature-sensitive mutations of gene 43 lead to rapid shut off of DNA synthesis in response to shifts from permissive to nonpermissive temperature (32, 59).

It would be desirable to determine the actual number of polymerase molecules which are required per replication growing point under normal growth conditions. An accurate determination is not possible from the data which is reported here since both qualitative and quantitative variations in gene 43 DNA polymerase activity were probably involved in the observed growth of the different T4 gene 43 *am* mutants in *su*⁻ hosts. It is interesting to note, however, that the results which were obtained with some of the mutants (summarized in Table 7) suggest that, normally, more T4 gene 43 polymerase is made than is required for maximal levels of DNA synthesis.

An examination of the growth responses of several T4 *am* mutants (Table 8 and unpublished results) suggests that all amber mutations are normally suppressible by translational ambiguity, but that the ability of an *am* mutant of an essential gene function to grow in response to this suppression depends on several factors

including the following:

(i) the efficiency of suppression. This can vary in the same bacterial host depending on the location of the mutated codon in the mRNA (49).

(ii) Translation of UAG to an amino acid may or may not result in an enzymatically active product. This is suggested by the experiment for Table 5 where some gene 43 *am* mutants failed to grow on certain UAG suppressors and where the growth of some mutants was temperature-sensitive on certain suppressors. It appears that mutants which responded well to most amber suppressors, also responded well to suppression by ambiguity. In the cases of *amE4311* and *amE4327*, the apparent weak response to ambiguity may be due to gene 43 products of low enzymatic activity rather than to misreading being specifically of very low efficiency at these two sites.

(iii) There is probably a certain threshold of active gene product concentration which has to be met or be exceeded for replication to occur. Products from different genes are probably required in different amounts. Our results suggest that very small amounts of the T4 gene 43 DNA polymerase and of the products of genes 42, 44, 56, and 62 are sufficient to support productive T4 DNA replication.

However, although some of these gene products play catalytic roles in replication their sites of action may or may not be the replication growing point. Other studies, for example, suggest that the products of T4 genes 43, 32, and 45 play roles in the repair of gaps which seem to be generated when phage DNA is replicated in the absence of the T4 gene 30 DNA ligase (31). This repair by the gene 43 polymerase is probably specific to replication since the enzyme does not appear to have a role either in recombination or in repair of UV-irradiated T4 DNA in vivo (53).

Finally, it should be emphasized that the growth properties of T4 *am* mutants can serve as tools for identifying suppressor mutations of *E. coli*. It should also be emphasized that the *E. coli* strain P3478 carries an *am* mutation in the *polA* gene and that some pol I is probably synthesized by these bacteria due to suppression by translational ambiguity. However, it is unlikely that the ability of P3478 to grow is dependent on the synthesis of pol I since *str*^r derivatives of P3478 which retain the *polA* mutation grow at the same rate as *str*^r *pol*⁺ bacteria in spite of the apparent loss of the ability to suppress by ambiguity.

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