Genetic analysis of the interaction between bacteriophage T7 DNA polymerase and *Escherichia coli* thioredoxin

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**ABSTRACT** Gene 5 protein of bacteriophage T7 is a nonprocessive DNA polymerase. During infection of *Escherichia coli*, T7 annexes the host protein thioredoxin for use as a processivity factor for T7 DNA polymerase. We describe here a genetic method to investigate the interaction between T7 gene 5 protein and *E. coli* thioredoxin. The strategy is to use thioredoxin mutants that are unable to support the growth of wild-type T7 phage to select for T7 revertant phage that suppress the defect in thioredoxin. A thioredoxin mutation that replaces glycine at position 74 with aspartic acid fails to support the growth of wild-type T7. This mutation is suppressed by six different mutations within T7 gene 5, each of which results in a single amino acid substitution within gene 5 protein. Three of the suppressor mutations are located within the putative polymerization domain of gene 5 protein, and three are located within the putative 3'-to-5' exconuclease domain. Each suppressor mutation alone is necessary and sufficient to confer the revertant phenotype.

The efficient polymerization of nucleotides at a replication fork is best accomplished by a highly processive mechanism of DNA synthesis. Processive DNA synthesis can lead to the incorporation of thousands of nucleotides in a single binding event between a DNA polymerase and a primer-template. In contrast, a completely nonprocessive DNA polymerase dissociates after the incorporation of a single nucleotide. In *Escherichia coli*, the DNA polymerase III holoenzyme consists of 10 polypeptides, with the β and γ subunits and the five-polypeptide γ complex being required for processive polymerase (1, 2). Processivity of the bacteriophage T4 DNA polymerase, the product of gene 43, is increased by three accessory proteins, the products of T4 genes 44, 62, and 45 (3, 4). The DNA polymerase of herpes simplex virus type 1 requires the viral UL42 gene product for processive DNA synthesis (5). The mammalian DNA polymerase, polymerase δ, interacts with the proliferating cell nuclear antigen (PCNA) and replication factor C (RF-C) to increase its processivity (6-9). The yeast analogs of mammalian PCNA and RF-C interact with yeast DNA polymerase III to increase the processivity of DNA synthesis (10, 11).

The DNA polymerase of bacteriophage T7, the subject of this communication, is the 80-kDa product of the viral gene 5 (12). The gene 5 protein has low processivity, dissociating from the primer-template after catalyzing the incorporation of 1-50 nucleotides (13). Upon infection, phage T7 annexes a host protein, as a processivity factor for the gene 5 protein (14, 15). *E. coli* thioredoxin binds tightly (Km = 5 nM) to T7 DNA polymerase in a 1:1 stoichiometry. Thioredoxin stabilizes the binding of gene 5 protein to a primer-template by 20- to 80-fold and increases the processivity of polymerization by 1000-fold (13, 16). The characteristics of the gene 5 protein–thioredoxin interaction—a high affinity, a 1:1 stoichiometry, and the ability to form a stable complex without an energy requirement—make it an attractive model for examining the mechanisms involved in processivity.

Thioredoxin, the 12-kDa product of the trxA gene of *E. coli*, contains two reversibly oxidizable cysteine residues and functions as a general protein disulfide oxidoreductase within the cell (17). The three-dimensional structures of oxidized (18) and reduced (19) thioredoxin are known. Oxidized thioredoxin does not interact with T7 DNA polymerase (20). One surface of the thioredoxin molecule is especially flat and hydrophobic, and it has been suggested that this surface of thioredoxin provides a binding area for interactions between thioredoxin and other proteins (21). Thioredoxin is also required for filamentous phage assembly (22).

To dissect the gene 5 protein–thioredoxin interaction, Huber et al. (23) examined nine mutant thioredoxins for their ability to interact with wild-type T7 gene 5 protein both in vivo and in vitro. In an in vitro reconstitution assay with gene 5 protein, mutant thioredoxins with alterations at either one or both active-site cysteine residues could restore nearly full polymerase activity, albeit requiring higher concentrations than wild-type thioredoxin. In vivo, these active-site mutants of thioredoxin support normal phage growth when present at sufficiently high levels within the cell. The observed dissociation constant, Kdiss, of the mutant thioredoxins for gene 5 protein increased between 3- and several thousandfold as compared with the observed dissociation constant of wild-type thioredoxin for gene 5 protein.

In the present study, we have isolated mutant gene 5 proteins that are altered in their interaction with *E. coli* thioredoxin. Our approach has been to isolate T7 revertants that can grow on *E. coli* cells harboring a mutant thioredoxin that does not support the growth of wild-type T7 phage. We propose that the positions of the parental (thioredoxin) and the suppressor (gene 5) mutations will define, at least in part, the sites of interaction between the two proteins or will permit the identification of biochemical properties affecting processivity.

**MATERIALS AND METHODS**

**Enzymes.** Restriction enzymes were purchased from New England Biolabs. T4 DNA ligase, T4 polynucleotide kinase, and ΔTag version 2.0 DNA polymerase (TaqDNA) were obtained from United States Biochemical. DNA Thermal Cycler 480 and AmpliTaq were obtained from Perkin-Elmer/Cetus. Native T7 DNA polymerase and Δ28 T7 DNA polymerase (24) were gifts from S. Tabor (Harvard Medical School, Boston).

**Nucleotides and Oligonucleotides.** [γ-32P]ATP and [α-32P]dATP were obtained from DuPont/NEN. Deoxy-
uracil-containing M13mGP5-2 DNA was a gift from S. Tabor (Harvard Medical School). Oligonucleotides, provided by A. Nussbaum (Harvard Medical School), were used for the construction of site-specific mutations within T7 gene 5. The amino acid substitution (V31 = Val-3 → Ile, etc.), the sequence of the oligonucleotides (the underlined base derivates from the wild-type base), and the wild-type base are shown from left to right: V31, AAT, ATG, ATC, ATT. TcR GAC A, G; V32A, CT TAC GCA AGC TAC CG, T; A45T, TAT CTG GAT ΔCG CTG GAA G, G; E319K, GAT ACC CGC ΔAG TAC GTT G, G; E319V, AT ACC CGC ΔTGC TAC GTT GC, A; Y490C, GG CTG TGT GTT GCT GA, A. Oligonucleotides for the polymerase-chain-reaction (PCR) amplification of the region of the E. coli chromosome containing the thioredoxin gene had the sequence: 5'-ACGGCGGAGCGCTGCGCAACATGAT-3' and 5'-CGAACGGGCAACACAGTAT-3'.

Bacterial Strains, Bacteriophage Strains, and Plasmids. The name of the strain or plasmid, the relevant characteristics (in parentheses), and the source or reference are shown from left to right: RK4349 (metEl63::Tn10J) Coli Genetic Stock Center no. 6403; A307 (HfrC, ΔtraA307) ref. 22; MR800 (F-, pcnB80) ref. 25; JH20 (F-, pcnB80, ΔtraA307) this work; SB2117 [F-, trxA7 (C32S, C35S)] this work; SB2111 [F-, trxA11 (G74D)] this work; SB2113 [F-, trxA13 (G92D)] this work; H1M3S21 (F-, supE, trxA*) laboratory collection; MV1190 (F+, supE; supports T7 growth to a limited extent; host cell for T7a3 cross) laboratory collection; bacteriophage T7trrx (a T7 phage which has a wild-type trxA gene inserted in its genome between genes I and 1.1.) S. Tabor; T7a3 (a T7 phage whose genes 1.1 and 1.2 have been replaced by genes 1.1 and 1.2 of phage T3; this hybrid phage grows on F+, Hfr, and F' strains of E. coli) ref. 23; 5-28, 5-309, and 5-196 (three strains of T7 phage, each harboring a different amber mutation in gene 5; all three strains were obtained from F. W. Studier) ref. 26; GP5A6AA [T7 with a two amino-acid residue deletion in gene 5 protein (Ser-122 and His-123)] ref. 24; GP5A6AA [T7 with a six amino-acid residue deletion in gene 5 protein (Lys-118 through His-123)] ref. 24; M13mGP5-2 (T7 gene 5 cloned in M13mp8) S. Tabor; P1Cop (a virulent mutant of P1 that is unable to form lysogens) S. Wurgler; plasmid pBR322trxA7 [the trxA7 gene (C32S, C35S) cloned in pBR322] ref. 22; pBR325trxA11 [the trxA11 gene (G74D) cloned in pBR325] ref. 22; pBR325trxA13 [the trxA13 gene (G92D) cloned in pBR325] ref. 22; pGP5-3 (a pBR322 derivative which contains a wild-type trxA gene) ref. 13; E. coli strains and bacteriophage T7-infected cells were grown in LB medium (27) at 37°C. M13 phage-infected cells were grown in 2X YT medium (27) at 37°C. T7 revertants were isolated at 37°C.

Genetic Manipulations. P1 transductions (27) and purification of E. coli chromosomal DNA (28) were as described. T7 suppressor mutations were mapped by marker rescue as follows. The revertant phages were grown within E. coli HMS231(pGP5-3), a E. coli strain harboring a plasmid containing a wild-type T7 gene 5 (13). The phages in the resulting lysate were plated at high dilution to produce single plaques. Individual plaques were picked, and the phages were screened for the inability to grow on E. coli SB211, a thioredoxin mutant that replaces the glycine residue at position 74 with an aspartic residue (22), which cannot support the growth of wild-type T7 phage. Phages which were unable to grow on the E. coli SB211 host represented wild-type recombinants that arose by a double-crossover event between the suppressor phage and the plasmid pGP5-3. The double-crossover replaced or rescued the suppressor mutation on the phage with its wild-type allele on the plasmid. The presence of wild-type recombinants in a high proportion of the phage lysate indicated that the suppressor mutation was located within gene 5.

DNA Sequence Determination. To locate the position of the revertant mutations within the T7 genome, T7 DNA was purified (29), and the DNA sequence was determined as described (30), except that annealing the sequencing primer with the double-stranded template was carried out by heating the primer-template mixture at 95°C for 3 min followed by cooling at 37°C for 30 min.

To screen individual plaques for the presence or absence of a particular nucleotide alteration during the construction of site-specific gene 5 mutants by in vitro mutagenesis, rapid sequence determination of T7 DNA from single plaques was carried out in three steps. First, a fragment (~1 kilobase (kb)) of the T7 genome from a single plaque was amplified in vitro by PCR using AmpliTag DNA polymerase (31). Second, the amplified product was purified by the GeneClean kit (Bio 101, La Jolla, CA). Third, the DNA sequence of the amplified and purified fragment of T7 genome was determined by cycle sequencing (32) using T3 primer.

Site-Specific Mutagenesis of T7 Gene 5. In vitro mutagenesis of T7 gene 5 was carried out as described (24). T7 gene 5 was cloned in M13, and site-directed mutagenesis was carried out by selection against the deoxyuracil-containing template strand (33). The M13 vector contains the desired gene 5 mutation, and a T7 phage, containing a gene 5 amber mutation, were used to coinfect the E. coli host MV1190. The resulting lysate was plated on a nonsuppressor strain of E. coli to select for T7 phages harboring the desired gene 5 mutation. The desired gene 5 mutation was transferred from the M13 vector into the T7 genome through a recombination event that rescued the gene 5 amber mutation from the parental T7 phage and, through the same recombinational event, incorporated the desired gene 5 mutation into the T7 genome.

RESULTS

Construction of a pcnB+, ΔtrxA E. coli F' Strain. E. coli thioredoxin mutants were constructed by first creating a F' strain that was pcnB+ and ΔtrxA. Any desired thioredoxin mutant can then be constructed simply by transforming this strain with a plasmid which harbors the appropriate mutant thioredoxin allele. The F' genotype is required, since bacteriophage T7 productively infects only female cells. The pcnB+ mutation, which reduces the copy number of pBR322 plasmids and its derivatives within a cell (25), was used because a high copy number of the mutant thioredoxin gene will create less stringent selection conditions (23). Specifically, a large intracellular concentration of the mutant thioredoxin gene will result in protein overproduction and functional defects. The ΔtrxA mutation removes the possibility of generating a wild-type thioredoxin gene by recombination between the plasmid and the bacterial chromosome.

E. coli F' pcnB+, ΔtrxA was constructed by successive P1 transductions. The initial P1 transduction was carried out to link an easily selectable marker, tetracycline resistance (Tc8), to the ΔtrxA gene of E. coli A307, an HfrC strain. The Tc8 marker facilitates the transfer of the ΔtrxA allele into other strains in genetic manipulations. The first P1 transduction moved the metEl63::Tn10 marker, which encodes Tc8, from E. coli RK4349 into E. coli A307 (ΔtrxA) by selecting for Tc8 transductants. The Tc8 transductants were screened for their inability to support the growth of phase T7/T3 (T7/T38), a T7 phage which can grow within male strains but still requires thioredoxin. The Tc8 and T7/T38 transductants were designated E. coli A307 (ΔtrxA, metEl63::Tn10). The second P1 transduction moved the Tc8 marker and the ΔtrxA allele from E. coli A307Tc into MR800, a female strain that harbors a pcnB mutation. Tc8 transductants were screened for their inability to support T7 (T78) growth and for their ability to support T7trrx5 (T7trrx5) growth. Phase T7trrx5 has a wild-type thioredoxin gene inserted into its genome and is able to grow within a thioredoxin-defective host cell.
T7R, and T7trxA55 transductants from the second P1 transduction were F-; pcnB-, and ΔtrxA; a single transductant, E. coli JH20, was chosen.

Confirmation of the pcnB- and ΔtrxA Alleles. The presence of the pcnB- allele in the E. coli JH20 genome was confirmed as follows. E. coli JH20 (pcnB-, ΔtrxA) and E. coli A307 (pcnB+, ΔtrxA) were each transformed with the same plasmid (pBR322trxA7), which encodes an ampicillin-resistance gene. We compared the ability of E. coli JH20(pBR322trxA7) and E. coli A307(pBR322trxA7) to grow on increasing concentrations of ampicillin. At an ampicillin concentration of 1 mg/ml, the number of viable cells of E. coli JH20(pBR322trxA7) was 10-4 times the number of viable cells of E. coli A307(pBR322trxA7). At lower ampicillin concentrations (0, 50, and 200 μg/ml), the number of viable cells of E. coli JH20(pBR322trxA7) was equivalent to the number of viable cells of E. coli A307(pBR322trxA7). Presumably, the inability of E. coli JH20(pBR322trxA7) to grow at high concentrations of ampicillin was a consequence of a lower plasmid copy number within the cell.

The presence of the ΔtrxA307 allele in the E. coli JH20 genome was confirmed as follows. Chromosomal DNA from E. coli JH20 and E. coli MR80 (the trxA+ isogenic parent of JH20) was prepared, and the genomic region that contains the thioredoxin gene was PCR amplified, using oligonucleotide primers that flanked the deletion. The upstream primer had a nucleotide sequence corresponding to a position between the rep and trxA genes of the E. coli genome (34). The downstream primer was complementary to a region that is adjacent to the 3' end of the trxA gene (35). The PCR products were separated by gel electrophoresis, and the DNA fragments were visualized by ethidium bromide staining. The PCR product from E. coli JH20 was approximately 800 base pairs (bp) shorter than the PCR product from E. coli MR80, and the predicted length of the deletion was 815 bp (22). The PCR products from E. coli JH20 and E. coli MR80 were analyzed physically by using the restriction enzymes Ava II and Bgl II. The restriction-fragment pattern matched the predicted restriction-fragment pattern (22, 36, 37).

Thioredoxin Mutants of E. coli. On the basis of earlier studies (22, 23), we constructed three strains of E. coli, each harboring a different alteration in the thioredoxin gene. E. coli JH20 (F-, pcnB-, ΔtrxA) was transformed with plasmids containing the mutant thioredoxin alleles trxA7, trxA11, and trxA13 to generate E. coli strains SB217, SB211, and SB2113, respectively. E. coli SB217, harboring the trxA7 allele of the thioredoxin gene, produces a mutant thioredoxin that has both active-site cysteine residues replaced with serine residues (C32S, C35S). Purified TrxA7 thioredoxin binds to gene 5 protein 1/60th as tightly compared with the binding of wild-type thioredoxin to gene 5 protein (23). The TrxA11 thioredoxin has a Gly-74 residue replaced by an aspartic residue (G74D) and binds to gene 5 protein approximately 1/200th as well as wild-type thioredoxin (23). TrxA13 thioredoxin, which replaces the Gly-92 with an aspartic acid (G92D), does not bind to gene 5 protein (23).

As shown in Table 1, wild-type T7 grows on the C32S, C35S thioredoxin mutant, E. coli SB217, as well as it grows on the wild-type thioredoxin host, E. coli MR80. This fact supports the earlier conclusion (23) that the thioredoxin function of thioredoxin is not necessary for its ability to function as a processivity factor. Consistent with previous studies (23), E. coli SB2113, the thioredoxin mutant which replaces Gly-92 with an aspartic acid (G92D), does not support the growth of wild-type T7 (Table 1). The most interesting thioredoxin mutant for the purpose of this study is E. coli SB2111, a strain in which the Gly-74 residue of thioredoxin is replaced by an aspartic residue (G74D). This thioredoxin mutant does not support wild-type T7 growth.

Table 1. Ability of thioredoxin mutants to support T7 growth

<table>
<thead>
<tr>
<th>Host cells</th>
<th>trxA allele</th>
<th>Phage</th>
<th>e.o.p.*</th>
</tr>
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<tbody>
<tr>
<td>MR80</td>
<td>WT</td>
<td>WT T7</td>
<td>1.0</td>
</tr>
<tr>
<td>SB217</td>
<td>trxA7</td>
<td>WT T7</td>
<td>0.4</td>
</tr>
<tr>
<td>SB2113</td>
<td>trxA13</td>
<td>WT T7</td>
<td>&lt;1 × 10^-9</td>
</tr>
<tr>
<td>SB2111</td>
<td>trxA11</td>
<td>WT T7</td>
<td>5 × 10^-4</td>
</tr>
<tr>
<td>MR80</td>
<td>WT</td>
<td>T7trx5</td>
<td>1.0</td>
</tr>
<tr>
<td>SB2113</td>
<td>trxA13</td>
<td>T7trx5</td>
<td>1.7</td>
</tr>
<tr>
<td>SB2111</td>
<td>trxA11</td>
<td>T7trx5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*e.o.p. (efficiency of plating) = number of plaques per ml on the indicated host cells divided by the number of plaques per ml on E. coli MR80 (trxA+).

WT, wild type.

However, at a frequency of 5 × 10^-4, T7 plaques appear that are able to grow on this G74D thioredoxin mutant (Table 1).

In earlier studies, Huber et al. (23), using a T7/T3 hybrid phage to enable productive infection of E. coli containing F factors, found that none of these three thioredoxin mutants supported T7/T3 phage growth at 37°C when the mutant thioredoxin gene was present in a single copy within the cell. Under these conditions, the efficiency of plating of the T7/T3 hybrid phage on a C32S, C35S thioredoxin mutant was 5 × 10^-4. The 1000-fold difference between the plating efficiency of the T7/T3 hybrid phage on a C32S, C35S thioredoxin mutant versus the plating efficiency of wild-type T7 phage on a C32S, C35S thioredoxin mutant is puzzling. The difference may be a result of the replacement of genes 1.1 and 1.2 of T7 with genes 1.1 and 1.2 of T3 in the hybrid phage, or it could be a result of the different host E. coli strains.

Isolation and Identification of T7 Suppressor Mutations. E. coli SB2111 (trxA (G74D)) was used to select 10 T7 suppressors. Each suppressor phage contains a spontaneously generated mutation that compensates for the thioredoxin defect. All 10 suppressor mutations were located by marker rescue between nucleotides 14309 and 16747 of the T7 genome, a region containing the carboxyl terminus of gene 4.7, all of gene 5, and the amino-terminal half of gene 5.3 (38).

The precise position of each suppressor mutation was determined by DNA sequence analysis (Table 2). Five of the mutant phages contained the same nucleotide alteration, which resulted in the replacement of glutamic acid at residue 319 of T7 gene 5 protein with lysine. These five suppressors (named 5-E319K) will be considered as a single isolate, since the 10 suppressor phages were not isolated independently. On the basis of the position of the suppressor mutations, we have separated the T7 suppressor phages into two classes (Table 2 and Fig. 1). The first class of T7 suppressors (5-V31, 5-V32A, and 5-A45T) contains mutations that reside within the amino-terminal half of gene 5 protein, a region which includes the putative 3'-to-5' exonuclease domain (24). The second class of suppressors (5-E319K, 5-E319V, and 5-Y409C) contains mutations that reside within the carboxyl-terminal half of gene 5 protein, a region which includes the putative polymerization domain (24).

Characterization of the T7 Gene 5 Mutants. All six strains of T7 suppressors, each strain harboring a different gene 5

<table>
<thead>
<tr>
<th>Suppressor</th>
<th>Nucleotide alteration</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-V31</td>
<td>G-C → A-T at bp 14359</td>
<td>Val-3 → Ile</td>
</tr>
<tr>
<td>5-V32A</td>
<td>T-A → C-G at bp 14447</td>
<td>Val-32 → Ala</td>
</tr>
<tr>
<td>5-A45T</td>
<td>G-C → A-T at bp 14485</td>
<td>Ala-45 → Thr</td>
</tr>
<tr>
<td>5-E319K</td>
<td>G-C → A-T at bp 15307</td>
<td>Glu-319 → Lys</td>
</tr>
<tr>
<td>5-E319V</td>
<td>A-T → T-A at bp 15308</td>
<td>Glu-319 → Val</td>
</tr>
<tr>
<td>5-Y409C</td>
<td>A-T → G-C at bp 15578</td>
<td>Tyr-409 → Cys</td>
</tr>
</tbody>
</table>
A Single Mutation Is Necessary and Sufficient to Confer the Suppressor Phenotype. To rule out the possibility that other uncharacterized mutations were also necessary to suppress the G74D thioredoxin mutation, we constructed, by oligonucleotide-directed mutagenesis, six strains of T7 phage, each strain harboring a single mutation corresponding to one of the suppressor mutations. Each phage strain was propagated, throughout its construction, on a host that produced only wild-type thioredoxin. When first exposed to the G74D thioredoxin mutant by plating on E. coli SB2111, all six phage strains grew with an e.o.m. of $5 \times 10^{-4}$. Therefore, in each case, the single mutation in gene 5 was necessary and sufficient to confer the suppressor phenotype.

DISCUSSION

The three-dimensional structure of T7 DNA polymerase is not known. However, the three-dimensional structure of the large Klenow fragment of E. coli DNA polymerase I is known (42). The Klenow fragment is folded into two distinct domains. The smaller, amino-terminal, domain contains the 3'-to-5' proofreading exonuclease activity of the enzyme (43). The larger, carboxyl-terminal, domain displays a large cleft postulated to bind the primer-template (42) and contains the putative deoxyribonucleoside 5'-triphosphate (dNTP)-binding site where polymerization occurs (44). Extensive amino acid sequence homology exists between the Klenow fragment of E. coli DNA polymerase I, T7 gene 5 protein (Fig. 1), and other DNA-dependent DNA polymerases (39, 45). The strongest homologies between the Klenow fragment and T7 gene 5 protein include residues that form the putative DNA-binding groove (39).

By tertiary structure inference, we can construct a speculative three-dimensional structure of gene 5 protein to position the suppressor mutations on this inference-generated structure. In the crystals of Klenow fragment, approximately 50 amino acid residues, lying at the tip of the “thumblike” protrusion over the DNA-binding crevice, are not well ordered (42). It has been postulated that the function of this subdomain is to close the cleft, reducing the dissociation rate of the DNA substrate (39). We speculate that the Glu-319 residue of gene 5 protein is located at the junction between the flexible domain and the “thumblike” protrusion that hovers above the DNA-binding crevice. Furthermore, we speculate that thioredoxin binds gene 5 protein at the edge of this crevice such that the two proteins together clamp the primer-template into position. This clamping action would account for the increased affinity of the gene 5 protein for a primer-template in the presence of thioredoxin (13, 16).

In the 5-E319V suppressor phage, the replacement of Glu-319 of gene 5 protein with a valine residue may suppress the thioredoxin defect by compensating directly for a structural alteration that was created by the replacement of Gly-74 of thioredoxin with an aspartic residue. In the wild-type complex, the side-chain atoms of Glu-319 of gene 5 protein may be physically adjacent to the hydrogen atom of the Gly-74 residue of thioredoxin. Ionic repulsion between the Glu-319 residue of the wild-type gene 5 protein and the Asp-74 residue of the mutant thioredoxin may prevent the growth of wild-type T7 on E. coli SB2111 [trxA (G74D)]. In the mutant complex of the 5-E319V suppressor phage, the putative glutamate–glutamate interaction in the wild-type complex would be replaced by a valine–aspartate interaction. Similarly, in the 5-E319K suppressor phage, the side-chain atoms of Lys-319 of gene 5 protein may be physically adjacent to the side-chain atoms of Asp-74 of thioredoxin. The mutant lysine–aspartate interaction may create a novel salt bridge between the two proteins.

Genetic studies (24, 41) and DNA sequence homologies (39, 40) suggest that the 3'-to-5' exonuclease activity of gene 5 protein resides within the amino-terminal half of the protein (Fig. 1). We suggest that three suppressor mutations (V31, V32A, and A45T) are located within the 3'- to 5'-exonuclease domain of T7 DNA polymerase. Because of the nature and location of the amino acid substitutions, we do not believe that these three suppressor mutations represent contact points between thioredoxin and gene 5 protein. It is tempting to speculate that these suppressor mutations alter the exonuclease activity of gene 5 protein and suppress the thioredoxin defect by increasing the processivity of the gene 5 protein, thereby obviating, to a limited extent, the requirement for thioredoxin. However, simply reducing the 3'-to-5' exonuclease activity of gene 5 protein is not sufficient to compensate for the Gly-74 → Asp defect in thioredoxin, because other T7 phages, containing mutations that are known to reduce the 3'-to-5' exonuclease activity of gene 5 protein, do not grow within E. coli SB2111 [trxA (G74D)]. Specifically, T7 phage GPS32AAA, harboring a two-amino-
acid deletion within gene 5 protein that decreases the 3'-to-5' exonuclease activity (24), does not grow within E. coli SB2111 (e.o.p. = 1 × 10^-5). Similarly, T7 phage GP5A6AA (24), carrying a six-amino-acid deletion of gene 5 protein that reduces the 3'-to-5' exonuclease activity to a greater extent, also does not grow within E. coli SB2111 (e.o.p. = 8 × 10^-5). We note that none of our T7 suppressors are bypass mutants, since they do not grow on an E. coli strain harboring a deletion of the thioredoxin gene.

Although the V31, V32A, and A45T suppressor mutations may, in fact, alter the exonuclease activity of gene 5 protein, we propose that they compensate for the thioredoxin defect by creating a conformational change in gene 5 protein. This conformational change could affect the ability of gene 5 protein to interact with other T7 replication proteins at the replication fork. The hypothesis that these suppressor mutations function by creating a conformational change in gene 5 protein is supported by the fact that all three of these suppressor mutations are not allele specific, as assayed by plaque formation. The fact that the S-E319V and E319K phage suppressors are also not allele specific seems, at first, to weaken the hypothesis that Glu-319 of gene 5 protein and Gly-74 of thioredoxin represent a direct contact point between the two proteins. However, these two mutations may be allele specific at a biochemical level; for example, the affinity of the mutant Val-319 and the mutant Lys-319 gene 5 proteins for the mutant Asp-74 thioredoxin may be greater than the affinity of either mutant gene 5 protein for the wild-type thioredoxin or for the active-site mutants of thioredoxin.

Again by tertiary structure inference based on sequence homology, we suggest that the Tyr-409 residue of gene 5 protein lies within a region of the polymerization domain which abuts the 3'-to-5' exonuclease domain. By homology-based inference, the Glu-319 residue and the Tyr-409 residue of gene 5 protein would be separated in space; therefore, tertiary structure inference leads to the conclusion that Glu-319 and Tyr-409 of gene 5 protein cannot both interact with Gly-74 of thioredoxin. We postulate that the Cys-409 suppressor mutation in gene 5 protein may function by altering the conformation of gene 5 protein.

If we reject the conceptual framework generated by tertiary structure inference based on sequence homology, then we must consider the possibility that the structure of T7 DNA polymerase at a replication fork may be significantly different from the crystal structure of the large Klenow fragment of E. coli DNA polymerase 1. If so, then the revertant mutations within T7 gene 5 may suppress the G74D thioredoxin defect by mechanisms different from the ones suggested above. These various possibilities can be addressed directly by purification of the gene 5 proteins that contain the suppressor mutations and characterization of their biochemical properties.

We dedicate this work to the memory of Salvador E. Luria. We are grateful to Alex Nussbaum for providing all the oligonucleotides used in this study, and we are indebted to Stan Tabor for helpful discussions throughout the course of this project. We thank S. Wurgler, F. Winston, and S. Tabor for critical reading of the manuscript. This work was supported by a John Stauffer Graduate Fellowship to J.S.H. from the Stauffer Charitable Trust and by grants from the U.S. Public Health Service (AI-00453) and the Department of Energy (DEG02-8ER660688).