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Role of the Acidic Carboxyl-terminal Domain of the Single-stranded DNA-binding Protein of Bacteriophage T7 in Specific Protein-Protein Interactions*

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The gene 2.5 single-stranded DNA (ssDNA) binding protein of bacteriophage T7 is essential for T7 DNA replication and recombination. Earlier studies have shown that the COOH-terminal 21 amino acids of the gene 2.5 protein are essential for specific protein-protein interaction with T7 DNA polymerase and T7 DNA helicase/primase. A truncated gene 2.5 protein, in which the acidic COOH-terminal 21 amino acid residues are deleted no longer supports T7 growth, forms dimers, or interacts with either T7 DNA polymerase or T7 helicase/primase in vitro. The single-stranded DNA-binding protein encoded by Escherichia coli (SSB) protein and phage T4 (gene 32 protein) also have acidic COOH-terminal domains, but neither protein can substitute for T7 gene 2.5 protein in vivo. To determine if the specificity for the protein-protein interaction involving gene 2.5 protein resides in its COOH terminus, we replaced the COOH-terminal region of the gene 2.5 protein with the COOH-terminal region from either E. coli SSB protein or T4 gene 32 protein. Both of the two chimeric proteins can substitute for T7 gene 2.5 protein to support the growth of phage T7. The two chimeric proteins, like gene 2.5 protein, form dimers and interact with T7 DNA polymerase and helicase/primase to stimulate their activities. In contrast, chimeric proteins in which the COOH terminus of T7 gene 2.5 protein replaced the COOH terminus of E. coli SSB protein or T4 gene 32 protein cannot support the growth of phage T7. We conclude that an acidic COOH terminus of the gene 2.5 protein is essential for protein-protein interaction, but it alone cannot account for the specificity of the interaction.

The gene 2.5 protein encoded by bacteriophage T7 is a single-stranded DNA (ssDNA) binding protein similar to the Escherichia coli SSB protein and the T4 gene 32 protein (1, 2). The gene 2.5 protein, like its counterparts in the E. coli and T4 phage systems, is essential for DNA replication and plays key roles in recombination and DNA repair (3–6). Biochemical studies have shown that gene 2.5 protein modulates several essential reactions of DNA replication and recombination; it interacts physically with T7 DNA polymerase to stimulate its activity (7) and with the T7 helicase/primase to increase the efficiency of RNA primer synthesis (8).

The ability of ssDNA-binding proteins to bind tightly to ssDNA is undoubtedly essential for their roles in replication, but their interactions with other replication proteins underlie their ability to coordinate reactions at a replication fork. Not surprisingly, the physical interactions of the ssDNA-binding proteins with other proteins show considerable specificity. In the case of T7 replication, neither E. coli SSB protein nor T4 gene 32 protein can substitute for gene 2.5 protein to support the growth of T7 phage (3). The inability of T4 gene 32 protein to replace T7 gene 2.5 protein in vivo can be explained biochemically. Whereas T7 gene 2.5 protein physically interacts with T7 DNA polymerase to stimulate its activity on ssDNA templates, T4 gene 32 protein has only a minor effect (7). Likewise, T7 gene 4 helicase/primase is unable to load onto ssDNA coated with T4 gene 32 protein, a reaction that occurs readily with T7 gene 2.5 protein-coated DNA (4). On the other hand, the inability of E. coli SSB protein to substitute for gene 2.5 protein is more difficult to explain. For example, E. coli SSB protein stimulates the activity of T7 DNA polymerase equally as well as does the gene 2.5 protein (7), and it interacts with T7 gene 4 protein to allow its entry onto ssDNA (4, 8). The only observable difference between the two proteins resides in their effects on primase activity. Whereas gene 2.5 protein increases the frequency of initiation of lagging strand synthesis by greater than 10-fold, E. coli SSB protein has no such effect (8). The possibility remains, of course, that there are other, as yet unrecognized, interactions involving the gene 2.5 protein, interactions that are specific for the gene 2.5 protein.

It is well documented that T4 gene 32 protein and E. coli SSB protein interact with their cognate proteins. For example, Formosa et al. (9) used affinity chromatography to demonstrate that T4 gene 32 protein physically interacts with at least 10 T4-encoded proteins including T4 DNA polymerase, dda helicase, and UvsX, UvsY proteins, which are involved in T4 DNA replication, recombination, and repair. By DNA synthesis assay and density gradient centrifugation, it was shown that E. coli SSB protein interacts with E. coli DNA polymerase II (10), exonuclease I (11), and a component of the primosome complex, protein n (12). Some preliminary results also suggest that E. coli SSB protein interacts with Rep and uvrD proteins (13, 14). The specificity of the protein-protein interactions involving T4 gene 32 protein and E. coli SSB protein has also been documented. E. coli SSB protein stimulates the polymerase activity of both E. coli DNA polymerases II and III, but not T4 DNA polymerase (10, 15–17), and T4 gene 32 protein specifically

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1 The abbreviations used are: ssDNA, single-stranded DNA; SSB, ssDNA-binding protein; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.

2 D. Kong and C. C. Richardson, unpublished results.
Chimeric Phage T7 Single-stranded DNA-binding Proteins

Numerous studies on the ssDNA-binding proteins have provided insight into the nature of the DNA binding domains and the domains involved in the specific interactions with their cognate replication proteins (19–21). Two regions of the T4 gene 32 protein, amino acid residues 9–21 and 253–275, are particularly susceptible to proteolytic cleavage (22–27). Cleavage at both sites leaves a core protein that retains its ability to bind to ssDNA. Loss of the NH2-terminal 9–21 residues abolishes cooperative binding of the protein to ssDNA. Deletion of the COOH-terminal region, which is relevant to our current studies, leaves a truncated T4 gene 32 protein that no longer interacts with either the T4 DNA polymerase or the gene 61 primase (24, 28–30). Williams et al. (30) have shown that the acidic COOH-terminal domain (23, 31, 42, or 62 amino acids) of E. coli SSB protein can be removed by limited proteolysis and that the resulting COOH-terminal truncated protein, like the truncated T4 gene 32 protein, retains its ability to bind to ssDNA. However, the question as to their ability to participate in protein-protein interactions remains unanswered. The similar arrangement of domains in E. coli SSB protein and T4 gene 32 protein suggests that the acidic COOH-terminal domains of both proteins are functionally homologous. The COOH terminus of the T7 gene 2.5 protein is also involved in protein-protein interactions. A truncated gene 2.5 protein, gene 2.5Δ21C protein, which lacks the COOH-terminal 21 amino acids, cannot support the growth of phage T7, and the purified mutant protein does not form dimers and does not interact with T7 DNA polymerase or T7 helicase/primase (31). The amino-terminal region of gene 2.5 protein contains a tyrosine-rich putative ssDNA binding motif shared by other ssDNA-binding proteins (32, 33).

Inasmuch as the COOH terminus of E. coli SSB protein, T4 gene 32 protein, and T7 gene 2.5 protein confers the ability of each protein to interact with its cognate replication proteins, the question arises as to the presence of a distinguishing structure or motif in this region. The COOH-terminal 25-amino acid sequence of each of the three proteins is shown in Fig. 1. The only distinctive feature of the COOH termini of all three proteins is the relatively high content of acidic residues. Of the carboxyl-terminal 21 residues, 6 in T4 gene 32 protein, 5 in E. coli SSB protein, and 15 in T7 gene 2.5 protein are acidic. No homology exists in this region among the three proteins.

To determine if the acidic COOH-terminal domain of the E. coli SSB protein, the T4 gene 32 protein, and the T7 gene 2.5 protein is solely responsible for the specificity of protein-protein interactions observed in vivo and in vitro, we have constructed chimeric ssDNA-binding proteins and examined their ability to support phage growth and to interact with other proteins in vitro. In this paper, we show that the COOH-terminal domain of either the E. coli or T4 or ssDNA-binding protein can substitute for the COOH-terminal domain of the T7 gene 2.5 protein. However, neither E. coli SSB protein nor T4 gene 32 protein in which the COOH-terminal domain has been replaced by the comparable domain of T7 gene 2.5 protein can substitute for T7 gene 2.5 protein in vivo.

EXPERIMENTAL PROCEDURES

E. coli Strains and Bacteriophages—E. coli strain HB101 (F− merB mrr hsdS20(rK−, mK + recA15 supE44 ara14 galK2 lacY1 proA2 rpsL20Sm(1)’ yll5 1 leu mtl1) was used as the host for plasmids pGP2.5–1, pGEM-gp2.5–ssb, and pGEM-gp2.5–32. Phage T7D2.5 constructed by Kim and Richardson (3) was prepared by amplifying this phage in E. coli W3110 (thyA− supr−) harboring the plasmid pGP2.5–1, which expresses the wild-type gene 2.5 protein. Growth of phage T7 or T7D2.5 on HB101 or W3110 in liquid or solid media was carried out as described (34). Burst sizes were determined as described previously (35, 36). Bacteriophage T4D2 was kindly provided by Dr. Ken Kreuzer (Duke University). The plasmids expressing wild-type gene 32 protein were provided by Dr. David P. Giedroc (Texas A & M University) and Dr. Youssif Shauho (Yale University).

Plasmids, DNA, and Proteins—Plasmid pGP2.5–1, which contains wild-type T7 gene 2.5 under the control of the tetracycline gene promoter, was constructed by Kim and Richardson (3). E. coli HB101 strain harboring the plasmid pGP2.5–1 was used for the growth of phage T7D2.5; in this phage, the entire gene 2.5 is deleted from the T7 genome. Plasmid vector pGEM-4Z harboring the promoter for T7 RNA polymerase was purchased from Promega and was used to construct the expression plasmids for the chimeric ssDNA-binding proteins gp2.5–ssb and gp2.5–32. The partial DNA fragments of T7 gene 2.5, T4 gene 32, and E. coli gene ssb were obtained by polymerase chain reaction from the genomic DNA of phage T4, T7, and E. coli strain W3110, respectively. All oligonucleotides were purchased from Integrated DNA Technologies, Inc.

T7 gene 2.5 protein and chimeric proteins gp2.5–32 and gp2.5–ssb were overexpressed in E. coli cells and purified to apparent homogeneity (>98%) as described by Kong and Richardson (4). The 63-kDa T7 gene 4 protein was purified by B. Beauchamp (Harvard Medical School) as described (37). T7 gene 5 protein-E. coli thioredoxin (1:1 complex) was purified and generously provided by S. Tabor (Harvard Medical School) as described (38). E. coli SSB, T4 gene 32 protein, and all restriction enzymes were purchased from Amersham Life Science, Inc. The specific activity of the COOH-terminal amino acids of T7 gene 2.5 protein and the COOH-terminal 24 amino acids of T4 gene 32 protein were obtained from Research Genetics, Inc. All nucleotides were purchased from Pharmacia Biotech Inc. All radioactive isotopes were purchased from Amersham Life Science.

Construction of Plasmids Containing Genes for Chimeric ssDNA-binding Proteins—If not otherwise indicated, DNA manipulations were performed according to the protocol described (39) or according to the instruction of the supplier. T7 gp2.5–ssb and T7 gp2.5–32 are chimeric proteins consisting of the NH2-terminal 209 amino acids from the NH2-terminal region of T7 gene 2.5 and the COOH-terminal 25 amino acids from the COOH-terminal region of T7 gene 2.5 protein or the COOH-terminal 22 amino acids from the COOH-terminal region of the T7 gene 32 protein, respectively. To construct the genes encoding the two chimeric proteins, we first inserted the polymerase chain reaction-amplified DNA fragments encoding the NH2-terminal 209 amino acids of T7 gene 2.5 protein and the ribosome binding site into the plasmid pGEM-4Z at PetI and XbaI sites. Two oligonucleotide primers, one containing a PetI site (5′-GGCCGGCTCTAGATCTGGAGATACGATACG-3′) and the other a XbaI site (5′-GGCGGTCGTCGACTGTATTG-3′), were used to amplify the T7 DNA sequence 9119–9787. The polymerase chain reaction fragment was purified by agarose gel electrophoresis, digested with restriction enzymes PetI and XbaI, and inserted into plasmid pGEM-4Z at PetI and XbaI sites to create plasmid pGEM-gp2.5–Δ22C. To construct the plasmid encoding T7 gp2.5–ssb, two oligonucleotides, 5′-CTAGAAGGGACAGCTGTCGTCGCTGCGGGATCTAGATGGTGTTGTCGTTTCGACAGC-3′ and 5′-AACTCTGACAGGAATGGTGTTGTCGTTTCGACAGC-3′, were designed to encode COOH-terminal 23 amino acids of T7 gene 32 protein, and annealed and inserted into pGEM-2.5–Δ22C at XbaI and EcoRI sites to yield plasmid pGEM-gp2.5–ssb. In a similar method, we also constructed plasmids pGEM-gp2.5–32 and pGEM-gp2.5–32. An XbaI recognition sequence, 5′-TCTAGA-3′, is present in both the chimeric genes and encodes amino acids Ser-Arg. The positions of Ser-Arg at chimeric protein gp2.5–ssb are identical to that found at the identical position in the COOH terminus of E. coli SSB protein. Therefore, the chimeric protein gp2.5–ssb has the NH2-terminal 209 amino acids from T7 gene 2.5 protein and the COOH-terminal 22 amino acids from E. coli SSB protein. The gene 5′–Δ22C chimeric protein gp2.5–32 has the NH2-terminal 209 amino acids from T7 gene 2.5 and the COOH-terminal 22 amino acids from T4 gene 32 protein. The Ser-Arg encoding by the XbaI recognition sequence is located at the junction. In a similar manner, we also constructed plasmids pGEM-gp2.5–32 and pGEM-gp2.5–32 which expresses chimeric proteins with the NH2-terminal 278 (or 253) amino acids of T4 gene 32 protein and the COOH-terminal 21 (or 60) amino acids of T7 gene 2.5 protein.
Chimeric Phage T7 Single-stranded DNA-binding Proteins

Plasmid pGEM-gpssb-2.5 expresses a chimeric protein having the NH2-terminal 115 amino acids of *E. coli* SSB protein and the COOH-terminal 60 amino acids of T7 gene 2.5 protein. In these three plasmids, two amino acids Ser-Arg encoded by XbaI recognition sequence 5'-TCTAGA-3' are present at the junction of the NH2-terminal and COOH-terminal domains.

**Overproduction and Purification of Chimeric Proteins gp2.5-ssb and gp2.5–32—** *E. coli* HBl101 cells carrying plasmid pGEM-gp2.5-ssb or pGEM-gp2.5–32 were grown at 30 °C in 500 ml of medium consisting of 2% tryptone, 1% yeast extract, 0.5% NaCl, 0.2% casamino acids, 40 mM K2PO4, pH 7.4, and 50 μg/ml ampicillin. At an *A*$_{600}$ of 1.0, bacteriophage aCS6 carrying the gene encoding T7 DNA polymerase was added to the culture to a multiplicity of infection of 8. Incubation was continued for an additional 3 h, and the cells were harvested and stored at −80 °C.

The chimeric proteins were purified by the procedure used for purification of wild-type gene 2.5 protein (4).

**Oligonucleotide Synthesis by 63-kDa T7 Gene 4 Primase—** Oligonucleotide synthesis by the T7 DNA primase (4). The reaction (10 μl) contained 40 mM Tris-Cl (pH 7.5), 10 mM MgCl2, 5 mM dithiothreitol (DTT), 50 mM potassium glutamate, 0.1 mM DTT, 0.5 mM concentration each of ATP, CTP, UTP, and GTP, 10 ng of M13mp18 ssDNA, 400 ng of ssDNA-binding protein. After incubation at 37 °C for 5 min to allow ssDNA-binding protein to bind to ssDNA M13mp18, 5 μCi of [α-32P]CTP and 100 ng of gene 4 protein were added to the reaction. After incubation at 37 °C for an additional 40 min, the reaction was stopped by the addition of 50 mM EDTA. Products of the reaction were analyzed by 25%, 1 M urea-PAGE as described previously (37).

**DNA Synthesis Catalyzed by T7 DNA Polymerase—** The T7 DNA polymerase assay was a modification of that described by Tabor and Richardson (40). The reaction mixture (40 μl) contained 250 ng of M13mp18 ssDNA primed by a 51-nucleotide oligonucleotide, 50 mM Tris-Cl, pH 7.5, 10 mM MgCl2, 5 mM DTT, 50 mM NaCl, 0.3 mM dNTPs, 0.4 μCi of [α-32P]CTP and 10 ng of T7 DNA polymerase, and the indicated amount of ssDNA-binding protein. The reaction was incubated at 37 °C for 3 min and stopped by the addition of EDTA to 50 mM, and then the reaction was transferred to Whatman DE81 filter. The DE81 filters were kept at room temperature to dry for 30 min and then washed with 90% ethanol. The filters were dried thoroughly, and the radioactivity released was measured by 25%, 1 M urea-PAGE as described previously (37).

**RESULTS**

**Construction of Chimeric ssDNA-binding Proteins—** A genetically altered T7 gene 2.5 protein, gene 2.5-2A1C protein, lacking the COOH-terminal 21 amino acid residues, no longer interacts with T7 DNA polymerase or helicase/primase and cannot support the growth of T7 phage lacking gene 2.5. These results suggest strongly that interaction between the gene 2.5 protein and other T7 DNA replication proteins are essential for T7 DNA replication and phage growth. Unresolved, however, is whether the acidic COOH-terminal domain confers specificity on the interaction with T7 DNA polymerase or the helicase/primase or merely provides a proper conformation or electrostatic charge to the protein while specificity of the protein-protein interactions resides elsewhere in the protein.

**CoOH-terminal 25-amino acid sequence of ssDNA-binding proteins: T7 gene 2.5 protein, T4 gene 32 protein, and E. coli SSB protein.**

The efficiency of coupling to the resin was 87% for T7 DNA polymerase bound to the affinity column. All fractions were analyzed by absorbance at 280 nm, and the presence of T7 DNA polymerase in each fraction was confirmed by SDS-PAGE. A standard curve of protein concentration versus log10 protein was determined by measuring the amount of radioactively labeled nucleotide synthesis by the T7 DNA primase (63-kDa gene 4 protein) (4). The reaction (10 μl) contained 40 mM Tris-Cl (pH 7.5), 10 mM MgCl2, 5 mM dithiothreitol (DTT), 50 mM potassium glutamate, 0.1 mM DTT, 0.5 mM concentration each of ATP, CTP, UTP, and GTP, 10 ng of M13mp18 ssDNA, 400 ng of ssDNA-binding protein. After incubation at 37 °C for 5 min to allow ssDNA-binding protein to bind to ssDNA M13mp18, 5 μCi of [α-32P]CTP and 100 ng of gene 4 protein were added to the reaction. After incubation at 37 °C for an additional 40 min, the reaction was stopped by the addition of 50 mM EDTA. Products of the reaction were analyzed by 25%, 1 M urea-PAGE as described previously (37).

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**Affinity Chromatography—** T7 gp2.5-2A1C, gp2.5-ssb, and gp2.5–32 were covalently coupled to Affi-Gel 10 following the manufacturer's instruction. The efficiency of coupling to the resin was 87% for T7 gp2.5-2A1C, 90% for gp2.5-ssb, and 91% for gp2.5–32. T7 DNA polymerase (0.2 mg) was mixed with 0.1 ml (drained volume) of the Affi-Gel 10 covalently linked to each of the three ssDNA-binding proteins and incubated for 15 min at 4 °C with gentle mixing. The mixture was transferred to a pipette tip column, and the column was washed with 1 ml of 20 mM Tris-Cl (pH 7.5), 0.1 mM EDTA, 0.1 mM DTT, and 10% glycerol. The column was further washed to remove any unbound T7 DNA polymerase. A step gradient (1 ml) of buffer A containing 50, 100, 150, 200, or 250 mM NaCl was used to elute T7 DNA polymerase bound to the affinity column. All fractions were analyzed by absorbance at 280 nm, and the presence of T7 DNA polymerase in each fraction was confirmed by SDS-PAGE.

**Amino acid sequence of ssDNA-binding proteins of both E. coli and phage T4 also have acidic COOH-terminal domains, but neither protein can substitute for T7 gene 2.5 protein for T7 phage growth (3).** These observations provide an approach to address the role of the COOH-terminal domain in providing specificity in protein-protein interactions. Consequently, we have substituted the COOH-terminal regions of these chimeric proteins for the corresponding COOH-terminal region of the T7 gene 2.5 protein. If the COOH-terminal regions of these proteins provide specificity for the protein-protein interactions, then neither chimeric protein should interact with T7 proteins to support the growth of phage T7. On the other hand, if the acidic COOH-terminal domain is needed only to provide a proper conformation or negative charge, then the chimeric proteins should function *in vivo* based on their similar acidic properties. If the former model is correct, the chimeric *E. coli* SSB protein or gene 32 protein containing the COOH terminus of gene 2.5 protein might support T7 growth.

The overall scheme for constructing the plasmids encoding the two chimeric proteins is presented in Fig. 2. T7 gene 2.5 lacking the coding region for the COOH-terminal 22 amino acids was inserted into pGEM-4Z. Subsequently, synthetic oligonucleotides encoding either the COOH-terminal 23 amino acids of *E. coli* SSB protein or the COOH-terminal 22 amino acids of phage T4 gene 32 protein were inserted at genetically engineered restriction sites adjacent to the COOH-terminal truncated coding sequence of gene 2.5 in plasmid pGEM-4Z to yield two plasmids, pGEM-gp2.5-ssb and pGEM-gp2.5–32, respectively. DNA sequence analysis confirmed that the two genes encode the two chimeric proteins consisting of the amino-terminal 209 amino acids of T7 gene 2.5 protein and either the 25 COOH-terminal amino acids of *E. coli* SSB protein or the 22 COOH-terminal amino acids of phage T4 gene 32 protein. These chimeric proteins are here referred to as gp2.5-ssb and gp2.5–32, respectively. To construct the chimeric plasmids, it was necessary to insert an XbaI recognition sequence, 5'-TCTAGA-3'. Since the XbaI sequence encodes the two-amino acid sequence Ser-Arg, this sequence appears at the chimeric junction of both chimeric proteins. In the case of gp2.5-ssb, the Ser-Arg sequence at this position is identical to that found in the COOH terminus of *E. coli* SSB protein (Fig. 1). Hence, the COOH-terminal 25-amino acid sequence in the chimeric protein is identical to that of the COOH terminus of *E. coli* SSB protein. The Ser-Arg sequence in gp2.5–32, however, leads to the insertion of an additional amino acid, Ser, between amino acids.
acids, Ser-Arg, are inserted at the junction of NH$_2$-terminal and COOH-terminal fragments. In the case of gp2.5–gp2.5–32 under the control of the T7 RNA polymerase promoter. Due to the presence of the XbaI recognition sequence 5'-TCTAGA-3', two amino acids, Ser-Arg, are inserted at the junction of NH$_2$-terminal and COOH-terminal fragments. In the case of gp2.5–gp2.5–32 at the indicated XbaI and EcoRI sites. The resulting plasmids pGEM-gp2.5-ssb and pGEM-gp2.5–32 express the two chimeric proteins gp2.5-ssb and gp2.5–32 under the control of the T7 RNA polymerase promoter. Due to the presence of the XbaI recognition sequence 5'-TCTAGA-3', two amino acids, Ser-Arg, are inserted at the junction of NH$_2$-terminal and COOH-terminal fragments. In the case of gp2.5-ssb, the two amino acids at this location are identical with those found at the identical position in the COOH terminus of E. coli SSB protein. Thus, the COOH-terminal 25 amino acids of gp2.5-ssb are from E. coli SSB protein.

### Table I

| ssDNA-binding protein | Plating efficiency | Size of plaque | Burst size |
|-----------------------|--------------------|----------------|-----------|
| gpssb                 | $<0.3 \times 10^{-9}$ | NA             | NA        |
| gp32                  | $<0.3 \times 10^{-9}$ | NA             | NA        |
| gp2.5–Δ21C            | $<0.3 \times 10^{-9}$ | NA             | NA        |
| gp2.5                 | 1                  | 98             | 38        |
| gp2.5-ssb             | 0.52               | 0.5            | 38        |
| gp2.5–32              | 0.22               | 0.1–0.2        | 7         |

* NA, data not available.

With gp2.5–32 was less efficient, with a burst size only 7% of that observed with gp2.5. Nevertheless, the gp2.5–32 protein supports the T7 growth at least 10$^2$-fold more efficiently than the gene 2.5–Δ21C protein (Table I).

We also constructed chimeric proteins in which the COOH terminus of E. coli SSB protein or T4 gene 32 protein was replaced with the COOH terminus of T7 gene 2.5 protein. Specifically, one chimeric protein contained the 115-amino acid NH$_2$-terminal sequence of E. coli SSB protein and the 60-amino acid residues 209 and 210 of gp2.5. We do not believe the additional serine residue significantly affects the conformation or biological activity of the chimeric protein gp2.5–32. As shown in Fig. 2, the ribosome binding site (rbs) of gene 2.5 is also present in the two expression plasmids, and a T7 RNA polymerase promoter lies just upstream of the genes to facilitate their expression.

### FIG. 2.

Construction of plasmids encoding the chimeric ssDNA-binding proteins, gp2.5-ssb and gp2.5–32. The construction contained two steps. For a detailed description of the plasmid construction, see “Experimental Procedures.” In the first step, the polymerase chain reaction-amplified DNA fragment harboring the ribosome binding site (rbs) and the coding sequence for the NH$_2$-terminal 209 amino acids of T7 gene 2.5 protein was inserted into pGEM-4Z at PstI and XbaI sites to yield pGEM-gp2.5-Δ22C. Two synthetic oligonucleotides, encoding either the COOH-terminal 23 or 22 amino acids (aa) of E. coli SSB protein or T4 gene 32 protein, respectively, were inserted into pGEM-gp2.5-Δ22C at the indicated XbaI and EcoRI sites. The resulting plasmids pGEM-gp2.5-ssb and pGEM-gp2.5–32 express the two chimeric proteins gp2.5-ssb and gp2.5–32 under the control of the T7 RNA polymerase promoter. Due to the presence of the XbaI recognition sequence 5'-TCTAGA-3', two amino acids, Ser-Arg, are inserted at the junction of NH$_2$-terminal and COOH-terminal fragments. In the case of gp2.5-ssb, the two amino acids at this location are identical with those found at the identical position in the COOH terminus of E. coli SSB protein. Thus, the COOH-terminal 25 amino acids of gp2.5-ssb are from E. coli SSB protein.

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Chimeric ssDNA-binding Proteins Support T7 Phage Growth—To determine if the COOH-terminal domain of E. coli SSB protein or T4 gene 32 protein could substitute for the COOH-terminal domain of T7 gene 2.5 protein to support T7 DNA replication, we transformed E. coli HB101 with the two expression plasmids, as described previously (3), thus demonstrating the inability of E. coli SSB protein to support T7 DNA replication. Likewise, T7 gene 2.5 protein lacking the 21 COOH-terminal amino acids cannot support growth of T7Δ2.5, whereas it grows essentially normally on E. coli strains expressing wild-type gene 2.5 protein. Somewhat to our surprise, both of the chimeric proteins, gp2.5-ssb and gp2.5–32, complemented the T7Δ2.5 mutation to provide plating efficiencies relative to wild-type gene 2.5 protein of 0.52 and 0.22, respectively. In the case of gp2.5-ssb, plaque size and burst size were only slightly reduced relative to complementation with gp2.5. Complementation
Fig. 3. Purification of the gp2.5-ssb and gp2.5–32 chimeric proteins. Overexpression and purification of the two chimeric proteins, gp2.5-ssb and gp2.5–32, are described under “Experimental Procedures.” The identities of the chimeric proteins are indicated. Lanes 1 and 2, gp2.5–32; cell extract and purified protein. Lanes 3 and 4, gp2.5-ssb, cell extract and purified protein.

Acid COOH-terminal region of T7 gene 2.5 protein, and two others contained either the 253- (or 279-) amino acid NH2-terminal sequence of T4 gene 32 protein and the COOH-terminal 60 (or 21) amino acids of T7 gene 2.5 protein, respectively. We found that none of these three chimeric proteins, when expressed in E. coli HB101, could support the growth of T7Δ2.5 phage (data not shown). Although these three chimeric proteins contain the COOH terminus of gene 2.5 protein, the overexpressed proteins did not interfere with the growth of wild-type T7 phage.

We conclude that the acidic COOH-terminal domain of either E. coli or phage T4 ssDNA-binding protein can substitute for the similar domain in T7 gene 2.5 protein to provide a functional protein in vivo. In view of these results, it is not surprising that placement of the COOH terminus of gene 2.5 protein on the larger NH2-terminal portion of the E. coli or T4 protein did not give rise to a chimeric protein capable of supporting T7 growth.

Chimeric ssDNA-binding Proteins Form Dimers—T7 gene 2.5 protein, T4 gene 32 protein, and E. coli SSB protein all form multimers. Gene 2.5 protein is a dimer in solution (1); T4 gene 32 protein exists as a monomer or dimer at low concentration but can form higher molecular weight species at higher concentrations (2). E. coli SSB protein, on the other hand, is a tetramer of four identical subunits (41). Removal of the COOH-terminal domain diminishes the stability of the tetramers (30). In the case of T7 gene 2.5 protein, the acidic COOH-terminal domain is not only essential for interactions with other replication proteins, but it is also essential for interactions with other gene 2.5 protein to form a dimer; T7 gene 2.5–21C protein, lacking the 21 COOH-terminal residues, exists as a monomer in solution although it is fully soluble and binds normally to ssDNA (31).

Each of the chimeric gene 2.5 ssDNA-binding proteins bearing either the COOH-terminal acidic domain of E. coli SSB protein or T4 gene 32 protein was purified to apparent homogeneity (>98%) from E. coli cells overexpressing each protein as described under “Experimental Procedures” (Fig. 3). The molecular weight of each of the chimeric proteins was estimated by gel filtration on a Superose 12 column. A plot of $K_v$ versus $log_{10} M_r$ was derived from the elution profiles of a number of protein standards and was used to estimate the native molecular weight of both chimeric proteins (Fig. 4). The molecular weight of both chimeric proteins is approximately 51,000, almost twice that of the denatured molecular weight of gp2.5–32 and gp2.5–ssb as determined by SDS-PAGE analysis (Fig. 3). We conclude that the acidic COOH-terminal residues contributed by T4 gene 32 protein or E. coli SSB protein allow the T7 gene 2.5 protein to dimerize at the protein concentration used in Fig. 4.

Physical Interaction between the Chimeric Proteins, gp2.5–32 and gp2.5–ssb, and T7 DNA Polymerase—T7 gene 2.5 protein physically interacts (Kd of 1.1 μM) with T7 DNA polymerase to form a 1:1 complex as measured by steady-state fluorescence emission anisotropy (7). In contrast, T7 gene 2.5–21C protein, lacking the COOH terminus, does not physically interact with T7 DNA polymerase (31). If protein-protein interactions involving the gene 2.5 protein are essential for T7 DNA replication, it seems likely that the chimeric proteins, since they support T7 phage growth, should physically interact with T7 DNA polymerase.

To determine if T7 DNA polymerase could interact directly with the chimeric gene 2.5 proteins, we examined its ability to bind to gp2.5-ssb and gp2.5–32 covalently coupled to a resin. Each purified chimeric protein was coupled to Affi-Gel 10 as described under “Experimental Procedures.” Each resin (0.1 ml of drained volume) was mixed with 0.2 mg of T7 DNA polymerase. After incubation for 30 min at 4 °C with gentle mixing, the mixture was loaded onto a 1-ml column and was washed with buffer A (20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 0.1 mM DTT, and 10% glycerol) to eliminate the unbound T7 DNA polymerase. In the absence of NaCl, greater than 90% of the T7 DNA polymerase bound to the resin. To elute the T7 DNA polymerase physically bound to the chimeric proteins, buffer A plus an increasing concentration of NaCl was used. As shown in Table II, T7 DNA polymerase was eluted from the gp2.5-ssb affinity column between 150 and 200 mM NaCl and from the gp2.5–32 affinity column between 50 and 100 mM NaCl. It has previously been reported that T7 DNA polymerase elutes from a wild-type gene 2.5 protein affinity column between 150 and 500 mM NaCl. As a control, the identical experiment was carried out with T7 DNA polymerase using Affi-Gel 10 that had been coupled to gp2.5–21C. In this case, T7 DNA polymerase eluted at less than 50 mM NaCl (Table II) as previously reported (31). The results show clearly that each of the chimeric proteins physically interacts with T7 DNA polymerase. Furthermore, the strength of the interaction can be correlated with the ability of the chimeric protein to support T7 growth. It therefore seems likely that the interaction of gene 2.5 protein
TABLE II
Physical interaction between T7 DNA polymerase and chimeric gene 2.5 protein

| Gene 2.5 protein | Concentration of NaCl required to elute T7 DNA polymerase from gp2.5 affinity column |
|------------------|---------------------------------------------------------------|
| gp2.5-Δ21C       | 0–50                                                          |
| gp2.5–32         | 50–100                                                        |
| gp2.5-ssb        | 150–200                                                       |

The physical interaction between T7 DNA polymerase and the COOH-terminal truncated or chimeric gene 2.5 proteins was examined by measuring the extent of retention of T7 DNA polymerase on an Affi-Gel resin, which had been coupled with gp2.5-Δ21C, gp2.5–32, or gp2.5-ssb. T7 DNA polymerase (T7 gene 5 protein and E. coli thioredoxin complex) was applied to the above ssDNA-binding proteins, coupled to Affi-Gel resin, and then eluted from the affinity column using a step gradient containing 0, 50, 100, 150, and 200 mM NaCl as described under “Experimental Procedures.” All fractions were analyzed by absorbance at 280 nm, and the presence of T7 DNA polymerase in each fraction was confirmed by SDS-PAGE.

FIG. 5. The effect of ssDNA-binding proteins on DNA synthesis by T7 DNA polymerase. The incorporation of [H]dGMP into DNA was determined as described under “Experimental Procedures.” The total amount of the four dNMPs incorporated into DNA is plotted as a function of the concentration of each DNA-binding protein. Each reaction mixture (40 μl) contained 0.25 μg of primed M13mp18 ssDNA, 0.01 μg of T7 DNA polymerase, and the indicated amount of the T7 gene 2.5 protein (gp2.5), gp2.5-ssb, gp2.5–32, or gp2.5-Δ21C.

with T7 DNA polymerase is important for DNA replication and phage growth.

Stimulation of T7 DNA Polymerase—T7 gene 2.5 protein, like E. coli SSB protein and T4 gene 32 protein (10, 18, 42), stimulates the activity of its cognate DNA polymerase (7, 38, 43–45). T7 gene 2.5-Δ21C on the other hand has no affect on T7 DNA polymerase activity, although it retains its ability to bind to ssDNA (30), a result that is not surprising in view of its inability to interact physically with T7 DNA polymerase as presented above. We have compared the ability of wild-type gene 2.5 protein, T7 gp2.5-Δ21C, and the two chimeric ssDNA-binding proteins to stimulate DNA synthesis catalyzed by T7 DNA polymerase on primed M13 DNA as a primer-template (Fig. 5). Such a comparison is of interest, since the four gene 2.5 proteins vary in their affinity for T7 DNA polymerase in the relative order gp2.5–32 > gp2.5-ssb > gp2.5–32 > gp2.5-Δ21C.

Wild-type gene 2.5 protein or each of the mutant proteins was added to DNA polymerase reactions containing a constant amount of T7 DNA polymerase, and DNA synthesis was measured. As previously reported, wild-type gene 2.5 protein significantly stimulates T7 DNA polymerase activity, whereas T7 gene 2.5-Δ21C protein has essentially no effect (Fig. 5). Both chimeric ssDNA-binding proteins stimulate T7 DNA polymerase activity to levels intermediate to these two extremes, with gp2.5-ssb having considerably more effect than gp2.5–32. Thus, the relative stimulation correlates precisely with the ability of each protein to bind to T7 DNA polymerase as well as to the efficiency of plating of T7 on strains expressing each of these proteins.

Stimulation of Gene 4 Primase—Gene 4 of phage T7 encodes a full-length 63-kDa protein and a second, co-linear protein, the 56-kDa gene 4 protein (37). The 56-kDa gene 4 protein, the T7 DNA helicase, is expressed from an internal ribosome binding site and start codon located 189 bases from the 5′-end of the gene 4 open reading frame (46). The 63-kDa gene 4 protein, by virtue of the additional 63 amino-terminal residues, in addition to being a helicase, is also a primase that catalyzes the template-directed synthesis of oligoribonucleotides, which in turn function as primers for T7 DNA polymerase (37, 47). The 63-kDa gene 4 protein can thus supply both helicase and primase activities at the replication fork, and hence it alone is sufficient to support T7 DNA replication and phage growth (48, 49). Gene 2.5 protein physically interacts with both molecular weight forms of gene 4 protein (7) and stimulates the synthesis of oligoribonucleotides by the 63-kDa primase (8). Again, the COOH-terminal domain of gene 2.5 protein has been shown to be essential for the interaction of gene 2.5 protein with gene 4 protein (4, 31). T7 gp2.5-Δ21C, lacking the COOH terminus, cannot bind to gene 4 protein (31), and it inhibits the gene 4 protein-mediated strand transfer reaction, presumably due to the inability of the gene 4 protein to bind to gp2.5-Δ21C coated DNA (4).

We have previously shown that the T4 gene 32 protein likewise inhibits the 4 helicase-mediated strand transfer reaction (5), since there is no interaction between the two proteins. In fact, T4 gene 32 protein alone prevents the binding of the T4 gene 41 helicase to ssDNA, since the gene 59 protein is required for the gene 41 protein to interact with the gene 32 protein (50, 51). Interestingly, the gene 4 helicase-mediated strand transfer reaction is not affected by E. coli SSB protein. Therefore, it was of interest to examine the ability of each of the two chimeric gene 2.5 proteins, one having the COOH terminus of T4 gene 32 protein and the other the COOH terminus of SSB protein, to interact with the gene 4 protein.

As a measure of the interaction of the ssDNA-binding proteins with gene 4 protein, we have examined the effect of each binding protein on template-directed oligoribonucleotide synthesis catalyzed by the 63-kDa gene 4 primase. In this assay, the template was M13 ssDNA, and oligoribonucleotide synthesis was measured by the incorporation of [α-32P] CMP into products that are detected by gel analysis. As shown in Fig. 6, T7 primase catalyzes the synthesis of di-, tri-, and tetrancleotides, and the reaction is stimulated by both T7 gene 2.5 protein (lane 5) and E. coli SSB protein (lane 7). The reaction is strongly inhibited by gene 2.5-Δ21C protein (lane 2) and T4 gene 32 protein (lane 6). Both chimeric proteins, gp2.5–32 (lane 3) and gp2.5-ssb (lane 4), however, stimulated the gene 4 primase, thus demonstrating an interaction of the chimeric proteins with the gene 4 protein.

Stimulation of T7 DNA Polymerase and Primase by Gene 2.5 Proteins

Although the COOH-terminal domains of all three ssDNA-binding proteins used in this study are sufficient to allow gene 2.5 protein to interact with other proteins, it is not known if the acidic COOH-terminal domains themselves directly dock with T7 DNA polymerase or T7 DNA helicase/primase. To address this point, we have examined the effect of synthetic peptides having the same amino acid sequence as that of the 21 COOH-terminal residues of
Chimeric Phage T7 Single-stranded DNA-binding Proteins

Experimental Procedures.

products is presented. The details of the procedure are described under "Experimental Procedures".

gene 2.5 protein or the 24 COOH-terminal residues of T4 gene 32 proteins on the ability of T7 gene 2.5 protein or the chimeric gp2.5–32 protein, respectively, to stimulate T7 DNA polymerase and T7 gene 4 primase (Fig. 7). The results show that the COOH-terminal peptides, even at relatively high molar ratios, do not impair the ability of the gene 2.5 protein or the gp2.5–32 protein to stimulate either reaction. The results suggest that the peptides do not bind to T7 DNA polymerase or to T7 DNA helicase/primase.

DISCUSSION

ssDNA-binding proteins such as the T7 gene 2.5 protein, the T4 gene 32 protein, and E. coli SSB protein discussed in this paper are involved in DNA replication, recombination, and repair (2). All three proteins are essential for phage or bacterial growth (2, 3). In vitro studies with these proteins have revealed that they modulate a large number of reactions involving proteins that interact with DNA. Herein lies a major difficulty in assigning relative importance to these in vitro effects, since a complete genetic analysis of the multiple effects has not yet been compiled. A second complication in addressing the essential role of these proteins in vitro is that both the ability of the protein to bind to ssDNA and its ability to interact with other proteins of DNA metabolism must be considered. One approach to this problem is to examine each property separately.

We have shown previously that the acidic COOH-terminal domain of the T7 gene 2.5 protein is required for several of the known protein-protein interactions involving this essential protein (31). Deletion of the COOH-terminal 21 acidic residues of gene 2.5 protein yields a truncated protein, T7 gene 2.5-Δ21C, that retains its ability to bind to ssDNA with the same affinity as does wild-type gene 2.5 protein. The retention of DNA binding is not surprising, since the domain putatively responsible for the binding, a domain found in a number of ssDNA-binding proteins (32), is present in the amino-terminal region of gene 2.5 protein. However, T7 gene 2.5-Δ21C protein no longer interacts with itself to form dimers or with T7 DNA polymerase or the T7 gene 4 helicase/primase to stimulate their activities (4, 31). Our findings that dimer formation is not

FIG. 6. Oligoribonucleotide synthesis by T7 63-kDa gene 4 primase. Oligonucleotide synthesis by the T7 DNA primase (63-kDa gene 4 protein) was determined by measuring the amount of radioactively labeled oligonucleotides after electrophoretic separation of the products (37). The reaction mixture contained M13 DNA template; ATP, UTP, GTP, and [α-32P]CTP; and 400 ng of the indicated ssDNA-binding protein. The autoradiograph of the electrophoresis gel separation of the products is presented. The details of the procedure are described under "Experimental Procedures".

FIG. 7. Effect of COOH-terminal peptides of T7 gene 2.5 protein and T4 gene 32 protein on stimulation of T7 DNA polymerase and primase by gene 2.5 proteins. A, T7 DNA polymerase. The effect of the COOH-terminal peptide (21 amino acids) of T7 gene 2.5 protein on the stimulation of DNA synthesis by T7 DNA polymerase was measured in a standard DNA polymerase assay containing gene 2.5 protein and the indicated amounts of the peptide. The reaction mixture is as described under “Experimental Procedures” except that 0.01 μg of T7 DNA polymerase, 1 μg of T7 gene 2.5 protein, and the indicated amounts of the gene 2.5 COOH-terminal peptide were present. After incubation for 3 min at 30 °C, the reaction was stopped by the addition of EDTA to 50 mM, and the amount of radioactively labeled deoxyribonucleotide incorporated into DNA was measured (dark bars). In the studies of the COOH-terminal peptide (24 amino acids) of the T4 gene 32 protein, reaction conditions were the same except that 1 μg of the chimeric gp2.5–32 and the indicated amounts of the COOH-terminal peptide of T4 gene 32 protein were used (diagonally striped bars). The molar ratios of the peptides to the ssDNA-binding proteins are indicated. B, T7 primase. The effect of the COOH-terminal peptide (21 amino acids) of the T7 gene 2.5 protein on the stimulation of primer synthesis by T7 gene 4 primase was measured in a standard gene 4 primase assay containing gene 2.5 protein and the indicated amounts of the peptide. The reaction mixture is as described under “Experimental Procedures” except that 0.01 μg of T7 DNA polymerase, 1 μg of T7 gene 2.5 protein, and the indicated amounts of the gene 2.5 COOH-terminal peptide were present. After incubation for 3 min at 37 °C, the reaction was stopped, and the products were analyzed by 25% PAGE in the presence of 1 M urea. When the COOH-terminal peptide (24 amino acids) of T4 gene 32 protein was examined, the indicated amounts of the COOH-terminal peptide of T4 gene 32 protein and 300 ng of the chimeric gp2.5–32 were used.
necessary for binding to ssDNA demonstrated the essential nature of protein-protein interactions; gene 2.5-Δ21C protein cannot support the growth of T7 phage lacking gene 2.5. Likewise, the loss of a physical interaction of gene 2.5-Δ21C with T7 DNA polymerase and the helicase/primase and a loss of its ability to stimulate their activities further emphasize the importance of these protein-protein interactions.

In the present study, we have attempted to address the specific role of the COOH terminus of gene 2.5 protein in protein-protein interactions. The approach we have used is based on the presence of a similar COOH-terminal domains in T4 gene 32 protein and in E. coli SSB protein (2). A number of studies have shown a similar separation of domains in the T4 and E. coli proteins. In E. coli SSB protein, the ssDNA binding domain has been shown to reside, at least partly, in the amino-terminal region (29, 30, 53–56). More important to the current work are a number of studies that implicate the acidic COOH-terminal domains of the T4, T7, and E. coli proteins in specific protein-protein interactions. In the case of T4 gene 32 protein, limited proteolysis has been used to generate three active fragments (23, 28, 54). Cleavage between residues 9 and 21 removes the NH₂-terminal region, producing gp32-B, while cleavage between residues 253 and 275 removes the acidic COOH-terminal A region, producing gp32-A. Cleavage at both sites results in gp32-(A + B). As expected from the above discussion, the B domain was implicated in cooperative ssDNA binding, but the acidic A domain was found to be responsible for interactions with other T4 replication proteins. Similar proteolytic studies have shown that the COOH-terminal domain of E. coli SSB protein is involved in stabilizing the tetrameric structure of the protein (30).

The COOH termini of all three of the prokaryotic ssDNA-binding proteins discussed above are highly acidic, and deletion of these COOH-terminal acidic residues eliminates their ability to interact with their cognate DNA replication proteins. This raises the question as to whether or not the specificity for these interactions resides exclusively within this domain. The lack of homology among the COOH-terminal domains of T4, T7, and E. coli ssDNA-binding proteins shown in Fig. 1 seems to imply their roles in specific protein-protein interaction in vitro and to account for the inability for one ssDNA-binding protein to substitute for another in vivo. In this study, we have used the direct approach of substituting the COOH-terminal domains of the T4 gene 32 protein and the E. coli SSB protein for the corresponding COOH-terminal domain of T7 gene 2.5 protein and likewise replacing the COOH-terminal domains of the former two proteins with the COOH-terminal domain of the T7 gene 2.5 protein. These chimeric proteins were then examined for their ability to support the growth of T7 phage and to interact with DNA replication proteins encoded by phage T7.

The chimeric proteins bearing the COOH-terminal domain of gene 2.5 protein were not able to support growth of T7, not a surprising result in view of the fact that the major portions of these three ssDNA-binding proteins are nonhomologous and are certain to have different tertiary structures. This result may further indicate that the NH₂-terminal region may have functions, in addition to its binding to ssDNA, such as in specific protein-protein interaction. Although no further studies were carried out with these chimeric proteins, it would be of interest to see if they could physically interact with T7 DNA polymerase and the gene 4 protein to stimulate their activities and to determine if they are monomers, dimers, or tetramers in solution.

Our results show that the acidic COOH-terminal domain of either T4 or E. coli SSB protein can substitute for the COOH-terminal region of gene 2.5 protein to provide for T7 DNA replication and phage growth, albeit slightly less efficiently compared with wild-type gene 2.5 protein. Thus, the COOH-terminal domain of gene 2.5 protein is essential for mediating protein-protein interactions, but the specificity for a functional interaction must reside elsewhere on the protein. This in vivo interpretation was confirmed in vitro by the demonstration that purified chimeric proteins physically interact with T7 DNA polymerase and helicase/primase and stimulate both the polymerase and the primase. Furthermore, the chimeric proteins readily form dimers in solution as does the wild-type gene 2.5 protein, a property that is dependent on the presence of the COOH terminus (31). This latter observation also suggests that, although the COOH terminus is necessary for dimerization, the determinant for dimer formation might reside elsewhere in the gene 2.5 protein. E. coli SSB protein is a tetramer in solution, and its COOH terminus has also been implicated in tetramer formation (30).

What precisely is the role of the acidic COOH-terminal domain of ssDNA-binding proteins? The fact that this domain is essential for interacting with other proteins but not for specificity suggests that it interacts with other residues within the protein to induce a conformational change that is required for protein interaction and multimer formation. Such a role would provide a number of mechanisms for modulating its affinity for replication proteins and perhaps for ssDNA to which it also binds. In fact, the removal of COOH termini of E. coli SSB protein and T4 gene 32 protein increases the helix-stabilizing ability of these two proteins (2, 30). Our observation that the synthetic COOH-terminal peptides of the ssDNA-binding protein do not compete with the gene 2.5 protein for binding with either T7 DNA polymerase or primase support the interpretation that the COOH terminus does not directly dock with these proteins. It is interesting that gene 2.5 protein is a dimer, a structure that is dependent on the presence of the COOH terminus, yet interacts with T7 DNA polymerase and presumably gene 4 protein as a monomer (7). This result implies that either the COOH-terminal domains of the two gene 2.5 proteins in the dimer contact one another and are thus not available to bind with other proteins or else that conformational changes in the protein dictate the specificity of binding. In the latter case, the dimer must dissociate in order for the COOH terminus of the gene 2.5 protein to mediate another conformational change suitable for binding to T7 DNA polymerase.

Our earlier results on helicase-mediated strand transfer suggested that the interaction of gene 2.5 protein with T7 gene 4 helicase was essential for binding of the helicase to gene 2.5 protein-coated ssDNA (4). The presence of T7 gene 2.5-Δ21C protein on ssDNA entirely prevented the gene 4 protein from entering the strand transfer reaction. In this same reaction, T4 gene 32 protein also inhibits whereas E. coli SSB protein does not. Likewise, T4 gene 32 protein only slightly stimulates T7 DNA polymerase, whereas E. coli SSB protein markedly stimulates the polymerase-catalyzed reaction (7). In the case of E. coli SSB protein, a physical interaction with the T7 replication proteins can be invoke, since E. coli SSB protein has been shown to physically interact with T7 DNA polymerase by sedimentation analysis (11). It may well be that T7 has evolved such that both its DNA polymerase and helicase can interact with E. coli SSB protein, since upon infection of E. coli there is an abundance of SSB protein. If so, then one must postulate other reactions involving gene 2.5 protein, reactions that are unique for gene 2.5 protein. Such alternatives may include the ability of gene 2.5 protein to facilitate homologous base pairing (4) and its role in establishing a functional replisome at the T7 replication fork.³

³ J. Lee and C. C. Richardson, unpublished results.
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