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Biochemical and Molecular Biological Studies on the DNA Replication of Bacteriophage T7

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Bacteriophage T7 gene 2.5 protein, a single-stranded DNA binding protein, has been implicated in T7 DNA replication, recombination, and repair. Purified gene 2.5 protein has been shown to interact with the phage encoded gene 5 protein (DNA polymerase) and gene 4 proteins (helicase and primase) and stimulates their activities. Genetic analysis of T7 phage defective in gene 2.5 shows that the gene 2.5 protein is essential for T7 DNA replication and growth. T7 phage that contain null mutants of gene 2.5 were constructed by homologous recombination. These mutant phage (T7Δ2.5) cannot grow in *Escherichia coli*. After infection of *E. coli* with T7Δ2.5, host DNA synthesis is shut off, and T7Δ2.5 DNA synthesis is reduced to less than 1% of wild-type phage DNA synthesis (Kim and Richardson, 1993, Proc. Natl. Aca. Sci. USA, 90, 10173-10177). A truncated gene 2.5 protein (GP2.5-Δ21C) deleted the 21 carboxyl terminal amino acids was constructed by *in vitro* mutagenesis. GP2.5-Δ21C cannot substitute for wild-type gene 2.5 protein *in vivo;* the phage are not viable and exhibit less than 1% of the DNA synthesis observed in wild-type phage-infected cells. GP2.5-Δ21C has been purified to apparent homogeneity from cells overexpressing its cloned gene. Purified GP2.5-Δ21C does not physically interact with T7 gene 4 protein as measured by affinity chromatography and immunoblot analysis. The mutant protein cannot stimulate T7 gene 4 protein activity on RNA-primed DNA synthesis and primer synthesis. These results suggest that C-terminal domain of gene 2.5 protein is essential for protein-protein interactions.

Key words : DNA replication, gene 2.5 protein, protein-protein interaction, mutagenesis

Introduction

Three proteins, T7 DNA polymerase (gene 5 protein), T7 helicase/primase (gene 4 protein), and *Escherichia coli* thioredoxin, account for the basic reactions of leading and lagging strand synthesis at the DNA replication fork of bacteriophage T7 (Richardson, 1983). The product of gene 5, a DNA polymerase, forms a tight 1 : 1 complex with the 12-kDa thioredoxin of *E. coli* to achieve processivity of DNA synthesis (Modrich and Richardson, 1975; Tabor et al., 1987). Gene 4 encodes two polypeptides of 63-kDa and 56-kDa, the latter resulting from an internal start site in the same frame as the former. The 63-kDa gene 4 protein has both primase and helicase activity, while the 56-kDa protein has helicase activity but is devoid of primase activity (Bernstein and Richardson, 1988; Notarnicola and Richardson, 1993). The 26-kDa gene 2.5 protein binds specifically to single-stranded DNA (binding constant of 2.5×10^6 M^{-1}) with a stoichiometry of approximately 7 nucleotides per monomer of gene 2.5 protein (Kim et al., 1992a). The gene 2.5 protein has been implicated in T7 DNA replication, recombination, and repair (Reuben and Gefter, 1973; Kim et al., 1992a, b). Recently, Kim and Richardson (1993) have shown by genetic analysis that gene 2.5 protein is indeed essential for T7 DNA replication and growth; after infection of *E. coli* with T7 phage lacking gene 2.5 protein, host DNA synthesis is shut off and phage DNA synthesis is reduced to less than 1% of phage DNA synthesis in wild-type T7-infected *E. coli* cells.

Gene 2.5 protein is a dimer of two identical subunits in solution (Kim et al., 1992a). In addition to

its ability to interact with itself, it also physically interacts with the T7 DNA polymerase (Kim et al., 1992 b). The interaction between gene 2.5 protein and the gene 5 protein/thioredoxin complex has been demonstrated by affinity chromatography and fluorescence emission anisotropy (Kim et al., 1992b). The complex contains one monomer each of gene 2.5 protein, gene 5 protein, and thioredoxin. In addressing the essential role of gene 2.5 protein *in vivo,* one must consider both the ability of the protein to bind to single-stranded DNA and to interact with other replication proteins. Gene 2.5 protein stimulates DNA synthesis catalyzed by T7 DNA polymerase and increases the processivity of polymerization on single-stranded templates (Kim et al., 1992b), a result consistent with a role of a single-stranded DNA binding protein (SSB) in removing secondary structures that impede DNA polymerase on single-stranded DNA. *E. coli* SSB protein, on the other hand, is even more effective in stimulating T7 DNA polymerase (Kim et al., 1992b), yet it cannot substitute for gene 2.5 protein *in vivo* (Kim and Richardson, 1993). One approach to determining the specific roles of protein-protein interactions involving gene 2.5 protein is to identify the domain of gene 2.5 protein involved in the interaction and to characterize mutant gene 2.5 proteins altered in this domain. The carboxyl terminus of gene 2.5 protein is highly acidic. This acidic region is thought to play a role in the interaction with other replication proteins (Burke et al., 1980). We have constructed a truncated form of the gene 2.5 protein lacking the acidic carboxyl-terminal 21 amino acid residues (Kim and Richardson, 1994). The purified deletion mutant of gene 2.5 protein retains its ability to bind to single-stranded DNA, but cannot interact with itself or with T7 DNA polymerase. Additionally, mutant gene 2.5 protein no longer stimulates DNA synthesis by the T7 DNA polymerase (Kim and Richardson, 1994). In this paper we show by *in vitro* mutagenesis and biochemical analysis, that (i) the carboxyl terminal domain of gene 2.5 protein is essential for T7 growth, (ii) the gene 2.5 protein interacts with the 56-and 63-kDa gene 4 proteins, resulting in stimulation of primase activity of gene 4 protein, and (iii) the carboxyl terminal mutant gene 2.5 protein cannot interact with gene 4 protein, resulting in no longer stimulatory effects on RNA-primed DNA synthesis by gene 4 protein.

Materials And Methods

Bacterial Strains, Plasmids, and Phages—Strains used in this experiment are listed in Table 1. *E. coli* HMS 262, AN1, and JH21 strains lack the chromosomal thioredoxin (trxA). T7Δ2.5::trxA was constructed by homologous recombination and has a deletion of gene 2.5 with an insertion of the *E. coli* trxA (thioredoxin) gene at the position of gene 2.5 (Kim and Richardson, 1993). Construction of pGP2.5-WT, which encodes wild-type gene 2.5 protein, has been described (Kim and Richardson, 1993). Gene 2.5 protein lacking the carboxyl terminal 21 amino acids (GP2.5-Δ21C) has been described (Kim and Richardson, 1994).

Table 1. *Escherichia coli* strains used in this study

Strains	Genetic markers
HMS157	F⁻recB21 recC22 sbcA5 endA gal thi sup
HMS174	F⁻hsdR rK12⁻ mK12⁺ recA1
HMS262	F⁻hsdR pro leu⁻ lac⁻ thi⁻ supE tonA⁻ trxA
JH21	F⁻pcnB80, ΔtrxA307
AN1	F⁻Δtrx307 metE::Tn10
HB101	F⁻Δ(mcrCmrr) leu supE44 ara14 lacY1 galK2 proA2 rpsL20(Str^r) xyl-5 mtl-1 recA13

Proteins and Other Materials—Wild-type gene 2.5 protein was purified to apparent homogeneity from *E. coli* cells overexpressing gene 2.5 protein as described (Kim et al., 1992a). T7 gene 2.5 Δ21C protein (GP2.5-Δ21C) was purified as described (Kim and Richardson, 1994). T7 DNA polymerase (T7 gene 5

protein and *E. coli* thioredoxin complex) was purified from cells overproducing both polypeptides (Tabor et al., 1987). T7 gene 4 proteins consisting of both 56-kDa and 63-kDa forms in equimolar amounts and 56-kDa (small form) alone were purified as described (Nakai and Richardson, 1988). Other enzymes were from United States Biochemical Corp. T7 [^{3}H] DNA (10cpm/pmol) (Hinkle and Chamberlin, 1972) and unlabeled T7 DNA (Richardson, 1966) were prepared as previously described. Single-stranded M13mp7 [^{3}H] DNA (45cpm/pmol) was prepared as described by Matson and Richardson (1983). Unlabeled nucleotides were from Pharmacia. [^{3}H] labeled nucleoside triphosphates were from Dupont-New England Nuclear. DE81 filter discs were from Whatman. Affi-Gel 15 was from Bio-Rad Laboratories.

Measurements of DNA synthesis—DNA synthesis was measured essentially as previously described (Saito and Richardson, 1981). *E. coli* cells used in Table 2 were grown with shaking at 30℃ in M9 CAA medium. At a cell density of 3×10^8 cells per ml the bacteria were infected with the indicated T7 phages at a m.o.i. of 7. At the indicated times, aliquots (0.2 ml) of the phage-infected cells were removed and placed in tubes containing 10μl of [^{3}H] thymidine (50 μCi/ml) in order to measure DNA synthesis. After 90 sec incubation at 30℃, growth was terminated by the addition of 3ml of cold 5% trichloroacetic acid. The acid-insoluble material was collected on GF/C filters (Whatman) and washed three times with 3ml of ice-cold 1M HCl and two times with 3ml of 95% ethanol. The acid-insoluble radioactivity was measured in a toluene-based solvent in a liquid scintillation counter.

Affinity Chromatography—wild-type gene 2.5 protein (2mg) and GP2.5-Δ21C (2mg) were coupled to Affi-Gel 15 as described (Kim et al., 1988; 1992b). The efficiency of coupling to the resin was greater than 90%. T7 gene 4 proteins (1mg/ml) were dialyzed against Buffer A (20mM Tris.HCl, pH 7.5, 0.1mM EDTA, 0.1mM DTT, and 10% glycerol), and concentrated by centrifugation through an Amicon Centricon 30 filter. The concentrated T7 gene 4 proteins (0.25ml, 0.5mg) was mixed with 0.25ml (drained volume) of the Affi-Gel 15 resin covalently linked to wild-type gene 2.5 protein and GP2.5-Δ21C, separately. After incubating the mixture for 20min at 4℃ with gentle mixing, the slurry was applied to a 1ml Pasteur pipette column. The column was washed with 2ml of Buffer A. T7 gene 4 proteins were eluted from the column with a step gradient of increasing NaCl concentration; each step (2ml) contained buffer A plus either 50mM, 100mM, 150mM, 200mM, or 250 mM NaCl. All steps were carried out at 4℃ and a flow rate of 1ml/h. Fractions of 250μl were collected, the absorbance at 280nm was measured, and aliquots were assayed for gene 4 enzyme activity. Affinity chromatography using BSA (5mg) as a control experiment was carried out using the same procedure described above.

Table 2. Plating efficiencies of T7 phages on various *E. coli* strains

Strains/Plasmid	Efficiency of Plating[1]	
	T7 (WT)	T7Δ2.5::trxA[2]
AN1	$<10^{-9}$	$<10^{-9}$
AN1/pGP2.5 (WT)	$<10^{-9}$	0.92
AN1/pGP2.5Δ21C	$<10^{-9}$	$<10^{-9}$
JH21	$<10^{-9}$	$<10^{-9}$
JH21/pGP2.5 (WT)	$<10^{-9}$	0.93
JH21/pGP2.5-Δ21C	$<10^{-9}$	$<10^{-8}$
HMS262	$<10^{-9}$	$<10^{-9}$
HMS262/pGP2.5(WT)	$<10^{-9}$	0.96
HMS262/pGP2.5-Δ21C	$<10^{-9}$	$<10^{-9}$
HMS174	1	$<10^{-8}$

[1]Efficiency of plating is calculated by dividing the number of plaque forming units on a given strain by the number of wild-type T7 plaque forming units on *E. coli* HMS174.
[2]T7Δ2.5::trxA is selected and amplified on *E. coli* JH 21/pGP2.5(WT) cells.

Enzyme Assays—The DNA polymerase assay

was performed as described (Kim et al., 1992b). The RNA-primed DNA synthesis assay for measuring primase activity of the 63 kDa gene 4 protein has been described (Mendelman and Richardson, 1992). The assay for primer synthesis by gene 4 protein was performed as described (Nakai and Richardson, 1988).

Other Methods—Plating efficiencies of T7 wild-type and T7Δ2.7::*trxA* phages on various *E. coli* strains were measured as follows. Bacterial strains were grown at a density of 2×10^8 cells per ml. 0.1ml dilution of various T7 phages in LB were mixed with 0.2 ml bacterial culture and 3ml of top agar, plated on LB or LB/ampicillin plates. All plating were carried out at 30℃. [^{3}H] Thymidine uptake into DNA after T7 phage infections on various *E. coli* strains has been described (Kim and Richardson, 1993). The protocols for immunoblot analysis of gene 4 proteins have been described (Mendelman and Richardson, 1992). Absorption spectra were recorded with a Shimadzu (Model UV-160) and Hewlett Packard (Model 8452 A) spectrophotometer.

Results

Functional Analysis of Carboxyl Terminal Domain of Gene 2.5 Protein

Growth of Phage T7—In order to examine the role of the acidic C-terminal domain of the T7 gene 2.5 protein, The coding sequence for the carboxyl terminal 21 amino acids of the protein was deleted (Kim and Richardson, 1994). A plasmid (pGP2.5-Δ21C) carries the mutant gene 2.5 protein (GP2.5-Δ21C), lacking the 21 carboxyl terminal amino acids. In order to determine if mutant gene 2.5 protein (GP2.5-Δ21 C) could support the growth of phage T7, we examined the ability of T7Δ2.5::trxA (see Kim and Richardson, 1993) to grow on strains in which GP2.5-Δ21C is expressed. T7Δ2.5::trxA has the entire gene 2.5 replaced by the thioredoxin gene (trxA) of *E. coli;* T7 Δ2.5::trxA phages are not viable and are defective in DNA synthesis (Kim and Richardson, 1993). Wild-type T7 and T7Δ2.5::trxA phages were plated on *E. coli* AN1 (trxA$^-$) that contains plasmids producing either wild-type gene 2.5 protein or GP2.5-Δ21C (Table 2). Growth of T7 phage in *E. coli* AN1 depends on the expression of the phage encoded *E. coli* thioredoxin gene since T7 requires thioredoxin and *E. coli* AN 1 lacks the chromosomal thioredoxin gene (trxA). As shown in Table 2, GP2.5-Δ21C does not complement T7Δ2.5::trxA phage (efficiency of plating $<10^{-8}$), whereas the plating efficiency of T7Δ2.5::trxA phage on cells containing wild-type gene 2.5 protein is normal. Other thioredoxin mutant strains, JH21 and HMS 262, also give similar results as shown in Table 2. We conclude that the acidic C-terminal domain of gene 2.5 protein is required for gene 2.5 activity *in vivo.*

DNA Synthesis—T7 gene 2.5 protein is essential for T7 DNA replication; in the absence of gene 2.5 protein, phage DNA synthesis is less than 1% of that observed in wild-type phage infected cells (Kim and Richardson, 1993). The kinetics of DNA synthesis after infection of *E. coli* HMS174/pGP2.5-WT and *E. coli* HMS174/pGP2.5-Δ21C with T7Δ2.5::trxA were compared with those obtained with a wild-type phage infection and T7Δ2.5::trxA infection of *E. coli* HMS174 (Fig. 1). As shown previously (Kim and Richardson, 1993), there is a decrease in DNA synthesis in HMS 174 cells after infection with T7Δ2.5::trxA, presumably due to the shut-off of host DNA synthesis and there is no detectable DNA synthesis observed at any time after infection. The defect in synthesis can be overcome by complementation with a plasmid encoding wild-type gene 2.5 protein. In striking contrast, however, the C-terminal deleted gene 2.5 protein (GP 2.5-Δ21C) cannot restore any detectable DNA synthesis in T7Δ2.5::trxA phage infected cells (Fig. 1).

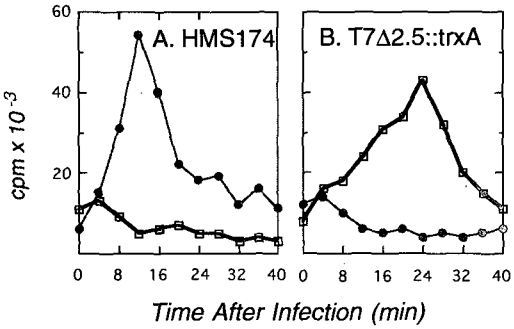

Fig. 1. Time courses of rates of DNA synthesis after infection.
The rates of DNA synthesis were measured (see text) at intervals after (A) infection of *E. coli* HMS174 cells with T7 wild-type (●) or T7Δ2.5::trxA (□) phages, (B) infection of *E. coli* HMS174/pGP2.5-WT (□) or HMS 174/pGP2.5-Δ21C (●) with TΔ2.5::trxA phages at a multiplicity of infection of 7 at 30℃. The values of each point are an average of data from two experiments.

Protein-Protein Interactions

Gene 2.5 Protein Affinity Chromatography—To determine whether T7 the primase/helicase (gene 4 protein) could interact directly with gene 2.5 protein using affinity chromatography, we examined its ability to bind to gene 2.5 proteins covalently coupled to a resin. Gene 2.5 proteins (wild-type and C-terminal deleted mutant) were coupled to Affi Gel at a concentration of 2.0mg of proteins/ml of resin as described under "Material and Methods." The Affi-Gel resin covalently linked to wild-type gene 2.5 protein (0.25ml of drained volume) was mixed with 0.5mg of T7 gene 4 proteins (56-kDa and 63-kDa proteins at equimolar ratio). After incubation for 20min. at 4℃ with gentle mixing, the mixture was applied to a column and eluted with Buffer A containing increasing concentrations of NaCl. In the absence of NaCl, greater than 90% of the T7 gene 4 proteins bound to the resin (Fig. 2A). Less than 10% of the T7 gene 4 proteins eluted with NaCl concentrations of 50 and 100mM. However, upon the addition of 150mM NaCl,~80% of the bound T7 gene 4 proteins eluted from the column. Eluted fractions showed the activity of gene 4 proteins assayed by RNA-primed DNA synthesis (Fig. 2) and were recognized by Western blot analysis using polyclonal antibody raised against T7 gene 4 small form (Fig. 3).

In the experiments described above, the form of the T7 gene 4 proteins applied to the column was the helicase/primase (an equimolar ratio of the 56-kDa and 63-kDa peptides; Nakai and Richardson, 1988), bound to the column in the absence of NaCl, and both peptides eluted together from the column in the presence of 150mM NaCl (Fig. 2A, 3A). Neither peptide was retained by the Affi-Gel resin coupled with BSA. Thus the affinity of T7 gene 4 proteins for the resin coupled to gene 2.5 protein is due to an interaction of T7 gene 4 proteins with gene 2.5 protein, and not to any nonspecific interactions between the gene 4 proteins and the resin. A preparation consisting solely of 56-kDa gene 4 protein, that has helicase activity but lacks primase activity (Bernstein and Richardson, 1988), also bound to the Affi-Gel resin coupled with gene 2.5 protein, and eluted at the same NaCl concentration (150mM) as the mixture. Thus T7 helicase/primase interacts with gene 2.5 protein, albeit with a weaker affinity than the interaction between gene 2.5 protein and T7 DNA polymerase (Kim et al, 1992b).

However, T7 primase/helicase does not bind to the Affi-Gel resin coupled to C-terminal deleted gene 2.5 (GP2.5-Δ21C) protein (Fig. 2B, 3B). Approximately 95% of the gene 4 protein was eluted from the column with Buffer A in the absence of NaCl. The remaining 5% was eluted with 50mM NaCl. This result with GP-Δ21C is in marked contrast to that of obtained with wild-type gene 2.5 protein, concluding that the acidic C-terminal region of gene 2.5 is responsible for protein-protein interaction with gene 4 protein.

Stimulation of T7 Primase Activity—GP2.5-Δ21C does not physically interact with T7 DNA polymerase, yet retains its ability to bind to single-stranded DNA (Kim and Richardson, 1994). We compared the ability of wild-type gene 2.5 protein and GP2.5-Δ21C to

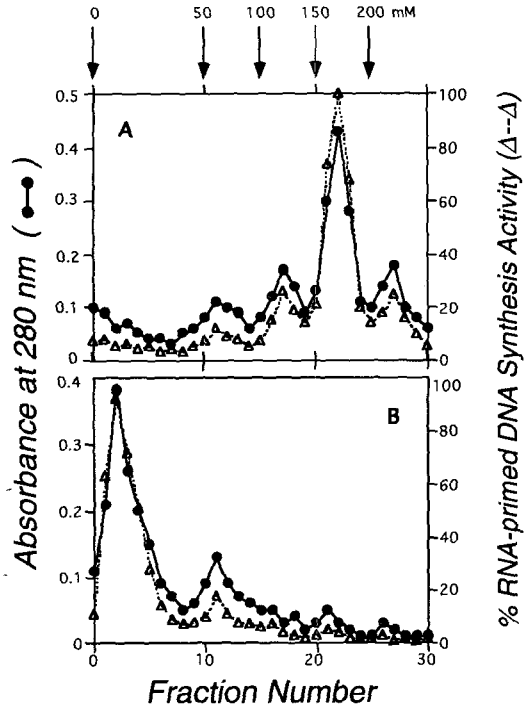

Fig. 2. Gene 2.5 Proteins affinity chromatography of T7 gene 4 proteins.
T7 gene 4 proteins were applied to Affi-Gel resin coupled to GP2.5 protein (A) or GP2.5-Δ21C protein (B) as described under "Materials and Methods." T7 gene 4 proteins were eluted from the column using 2-ml step gradients containing 0, 50, 100, 150, and 200mM NaCl (top arrows). 400μl fractions were collected, the absorbances at 280nm were determined (●), and aliquots were assayed for RNA-primed DNA synthesis (Δ). T7 gene 4 proteins eluted from Affi-Gel resin coupled to GP2.5 protein at 150mM NaCl (A) and GP2.5-Δ21C protein at 0mM NaCl (B), respectively.

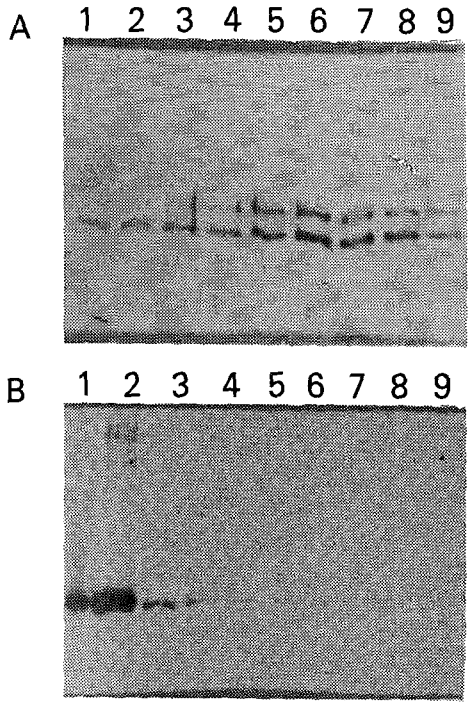

Fig. 3. Western Blot Analysis of gene 4 proteins after affinity chromatography.
T7 gene 4 protein (both 63 kDa and 56 kDa) was chromatographed to Affi-Gel resin coupled to GP2.5 protein (A) or T7 gene 4 small form (56 kDa) to GP2.5-Δ21C protein (B) as described under "Materials and Methods." Aliquots (10~20μl) from eluted fractions were treated with loading buffer (60mM Tris-HCl, pH 6.8, 1% β-mercaptoethanol, 3% sodium dodecyl sulfate (SDS), 10% glycerol, and 0.01% bromophenolblue), heated to 95℃ for 5 min, and loaded onto a 10% SDS-polyacrylamide gel. The procedures for transfer of proteins to nitrocellulose membrane and detection of gene 4 protein by immunoblot analysis have been described (Mendelman and Richardson, 1992). (A), lanes represent T7 gene 4 protein (63-kDa and 56-kDa) from Affi-Gel resin coupled to wild-type GP2.5 protein; eluted fractions are 50mM (lane 1-2), 100mM (lane 3-4), 150mM (5-7), 200 mM (8-9) of NaCl. (B), lanes represent gene 4 small form (56-kDa) eluted from Affi-Gel resin coupled to GP2.5-Δ21C protein; 0mM (lane 1-2), 50mM (lane 3-4), 100mM (5-6), 150mM (7-8), 200mM (9) of NaCl.

stimulate RNA-primed DNA synthesis by gene 4 protein and T7 DNA polymerase as described under "Material and Methods" (Fig. 4). Wild-type or mutant gene 2.5 protein was added to a constant amount of T7 gene 4 protein and RNA-primed DNA synthesis was measured. As shown in Fig. 4, wild-type gene 2.5 protein is very effective in stimulating RNA-primed DNA synthesis, while GP2.5-Δ21C does not stimulate T7 gene 4 protein activity significantly. RNA-primed DNA synthesis, when used to measure gene 4 primase activity, measures the amount of DNA synthesis initiated by primer synthesis. An important effect to consider is the ability of the gene 2.5 protein to

promote strand-displacement synthesis because strand-displacement DNA synthesis cannot be promoted by the gene 4 protein (Engler and Richardson, 1983). In the presence of gene 2.5 protein, it is possible that strand-displacement DNA synthesis beyond the RNA primers may take place, promoting processive strand-displacement synthesis by T7 DNA polymerase. Moreover, T7 gene 2.5 protein also stimulates CMP incorporation (gene 4 primase activity) by 4-fold whereas C-terminal truncated gene 2.5 protein (GP 2.5-Δ21C) is much less potent stimulating primer synthesis (Fig. 5). This result suggests that T7 wild-type gene 2.5 protein stimulates RNA-primed DNA synthesis not only by stimulating primase activity but also by promoting efficient usage of primers by T7 DNA polymerase and that C-terminal region of gene 2.5 protein is essential for stimulation of T7 primase activity.

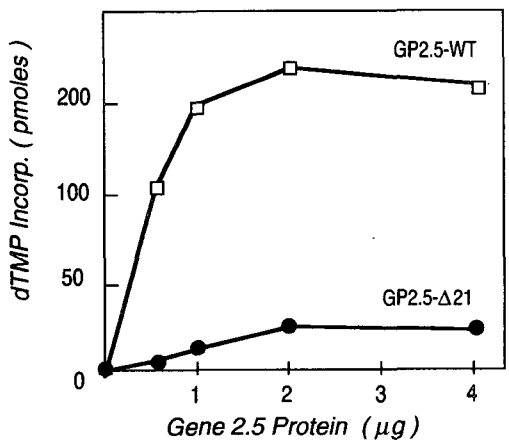

Fig. 4. Effect of GP2.5 Proteins on RNA-primed DNA synthesis by T7 gene 4 proteins.
The incorporation of [^{3}H]dTMP into DNA was determined as described under "Materials and Methods." Each reaction mixture (50μl) contained 50mM NaCl, 0.3mM each of [^{3}H]dTTP (85cpm/pmol), dATP, dGTP, dCTP, CTP, and ATP, 1.0μg of M13mp6 DNA, 125ng of T7 DNA polymerase (27 nM), 21ng of T7 gene 4 protein (7nM), and the indicated amounts of T7 wild-type gene 2.5 protein (□), GP2.5-Δ21C (●). Incubation was at 30℃ for 20min.

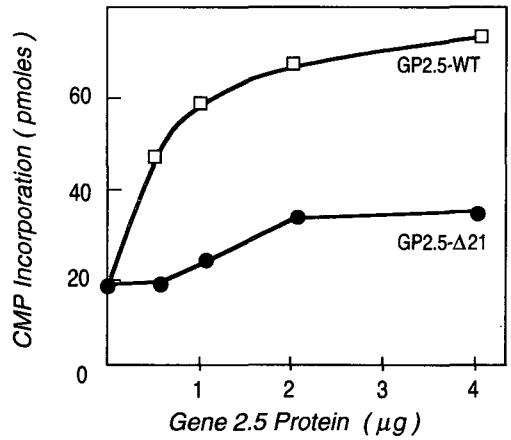

Fig. 5. Stimulation of the gene 4 primase activity by GP2.5 Proteins.
Reaction mixtures (25μl) contained reaction buffer 1mM TTP, 0.3mM ATP 50μM [α-^{32}P] CTP (8000cpm/pmol), 0.5μg of M13 DNA, 42ng of T7 gene 4 protein (14nM), and the indicated amounts of T7 wild-type gene 2.5 protein (□), GP2.5-Δ21C (●). Incubation was at 30℃ for 20 min. The products of the reaction were treated with calf intestine phosphatase, absorbed to Norit, dried, and counted in a noaqueous liquid scintillation mixture as described (Nakai and Richardson, 1988).

Discussion

Author has recently shown that T7 phages with a deletion of gene 2.5 do not grow in *E. coli* and they are defective in DNA replication (Kim and Richardson, 1993). The essential nature of gene 2.5 protein for T7 growth and DNA synthesis could be explained for its specific interaction with other phage encoded replication proteins, and for its role in recombination and repair. Biochemical studies on the purified gene 2.5 protein have previously demonstrated its physical interaction with T7 DNA polymerase by affinity chromatography and fluorescence emission anisotropy analysis (Kim et al., 1992b). The one to one complex is stable at moderate ionic strength but dissociates when the ionic strength of the medium is increased above 250mM. T7 gene 2.5 protein stimulates the

activity of T7 DNA polymerase (gene 5 protein/thioredoxin complex) on single-stranded DNA templates (Kim et al., 1992b).

In this study it has been evaluated that gene 2.5 protein physically interacts with another replication protein, the gene 4 proteins of T7 that provide for helicase and primase function. The specific interaction between gene 2.5 protein and T7 gene 4 proteins was detected by affinity chromatography. Furthermore, T7 wild-type gene 2.5 protein greatly increases the rate of initiation of RNA-primed DNA synthesis and primer synthesis not only by stimulating primase activity but also by promoting efficient usage of primers by T7 DNA polymerase. However, the reason for these interactions in T7 is not known in detail. This effect can partly account for the increased initiation rate of RNA-primed DNA synthesis. The specific interaction reflects a requirement for a highly ordered structure at the replication fork to coordinate leading-and lagging-strand DNA synthesis.

In order to gain insight into the role of the protein-protein interactions discussed above, the domain of gene 2.5 protein likely to be involved in these interactions was deleted. We deleted 21 amino acids from the carboxyl terminus of gene 2.5 protein, a highly acidic domain thought to be involved in protein-protein interactions in other single-stranded DNA binding proteins (see Chase and Williams, 1986). The truncated gene 2.5 protein, GP2.5-Δ21C can not substitute for wild-type gene 2.5 protein *in vivo*.

In view of the effect of the carboxyl terminal deletion on gene 2.5 protein, we were particularly interested in determining if the truncated gene 2.5 protein still retained the ability to interact with T7 gene 4 protein or stimulate its activity. The purified truncated protein does not physically interact with T7 gene 4 protein. T7 gene 4 protein does not bind to the Affi-Gel resin coupled to C-terminal deleted gene 2.5 protein, demonstrating that C-terminal acidic domain is important for physical interaction between gene 4 protein and gene 2.5 protein. Additionally, wild-type gene 2.5 protein is very effective in stimulating primer activity, while GP2.5-Δ21C does not stimulate primase activity of T7 gene 4 protein significantly. This result suggests that C-terminal region of gene 2.5 protein is essential for protein-protein interactions with gene 4 proteins and for stimulation of T7 primase activity. Results from the studies on the DNA replication of bacteriophage T7 using biochemical and molecular biological techniques will be applied to investigate on the replication system of marine microorganisms.

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Bacteriophage T7의 유전자 복제기작에 관한 생화학적, 분자생물학적 특성 연구

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본 연구에서는 유전자 복제기작을 생화학적, 분자생물학적 방법을 사용하여 bacteriphage T7을 대상으로 연구하였다. Bacteriophage T7의 유전자 복제, 재조합, 수선시 필수 단백질로 작용하는 gene 2.5 단백질의 생체내 기능에 대한 유전학적 연구와 단백질을 분리 정제하여 복제 단백질들과의 상호작용에 대한 연구를 수행하였다. 연구결과 gene 2.5 단백질은 DNA 복제시 필수 구성단백질로 작용하며, 복제과정에서 유전자 복제에 관여하는 핵심 단백질들인 DNA polymerase, helicase/primase와 직접 단백질-단백질 상호 협동 작용을 하는 것을 증명하였다. 특히 gene 2.5 단백질의 C-terminal domain이 절편된 변이체의 경우 복제 단백질들과 상호작용이 결여 되었다. 따라서 C-terminal domain이 gene 2.5 단백질의 기능에 필수적으로 관여함을 입증하였다.