An N-terminal fragment of the gene 4 helicase/primase of bacteriophage T7 retains primase activity in the absence of helicase activity

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ABSTRACT Primase and helicase activities of bacteriophage T7 are present in a single polypeptide coded by gene 4. Because the amino terminal region of the gene 4 protein contributes to primase activity, we constructed a truncated gene 4 encoding the N-terminal 271-aa residues. The truncated protein, purified from cells overexpressing the protein, is a dimer in solution; the full-length protein is a hexamer. Although the fragment is devoid of DTTase and helicase activities, it catalyzes template-directed synthesis of di-, tri-, and tetrnucleotides. The rates for tetraribonucleotide synthesis and for dinucleotide extension on a 20-nucleotide template are similar for the full-length and truncated proteins. However, the activity of the primase fragment is unaffected by DTT whereas the primase activity of the full-length protein is stimulated >14-fold. The primase fragment is defective in the interaction with T7 DNA polymerase in that primer synthesis cannot be coupled to DNA synthesis.

The replisome of bacteriophage T7 is composed of relatively few proteins. The multifunctional gene 4 protein encoded by the phage exemplifies this economical use of genetic information. Gene 4 encodes two collinear proteins from separate in-frame translational start sites, a 63-kDa and a 56-kDa protein. The smaller protein translocates 5' to 3' on single-stranded DNA (ssDNA) and unwinds duplex DNA (helicase activity) in a reaction coupled to the hydrolysis of a nucleoside triphosphate (1–3). The larger 63-kDa protein has all of the activities found in the 56-kDa protein and, in addition, catalyzes the template-directed synthesis of oligoribonucleotides (primase activity) (4–6) for use as primers by T7 DNA polymerase (7, 8). The N-terminal 63-aa residues are not present in the 56-kDa protein, and a cysteine zinc finger required for interaction with primase recognition sites (9–11). The presence of helicase and primase activities in the 63-kDa protein simplifies reconstruction of the T7 replisome (12), but the competitive effects of multiple activities have complicated the characterization of the DNA primase.

The identification of domains within the 63-kDa gene 4 protein is one approach to obtaining a primase protein without helicase activity. The zinc finger essential for primase activity is located in the unique 63-residue N terminus of the protein, but it is not sufficient for primase activity. The 63-aa fragment is devoid of primase activity, and the 56-kDa protein catalyzes the synthesis of random diribonucleotides (9). The nucleotide binding site essential for helicase binding to ssDNA and for translocation is located near the C terminus (13, 14). Other segments essential for functional helicase, one for hexamer formation and one for interaction with the polymerase, are also located near the C terminus. Limited proteolysis of the 63-kDa protein results in three major cleavages (15, 16). Using endoproteinase Glu-C, one cleavage, at Glu-52, releases a small N-terminal fragment containing the zinc finger, and a second cleavage releases a 3-kDa fragment from the C terminus (15). A third cleavage, at Glu-219, gives rise to two large fragments, of 20 and 33 kDa. On the basis of this cleavage site, the helicase domain has been assigned to the two C-terminal fragments (15). Likewise, based on sequence homology with other primases (17), the primase domain can be assigned to the large N-terminal fragment plus the small zinc finger fragment.

If the helicase and the primase reside in these separate domains, then the corresponding truncations of gene 4 should produce monofunctional helicase and primase proteins. Bird et al. (15) have confirmed this prediction by showing that the C-terminal domain is an active helicase. This helicase fragment can be expressed from a plasmid containing gene 4 but lacking the region predicted to encode the primase (15). We have constructed a plasmid encoding the N-terminal 271-aa fragment corresponding to the primase domain, after elimination of the internal start codon for the 56-kDa gene 4 protein (18, 19), and we describe the properties of a gene 4 fragment lacking helicase activity but retaining primase activity.

MATERIALS AND METHODS

Reagents and Strains. Oligonucleotides were obtained from Integrated DNA Technologies (Corralville, IA), and M13 ssDNA was from New England Biolabs. The 56-kDa and 63-kDa gene 4 proteins (13) and the T7 gene 2.5 protein (20) have been described. The 63-kDa gene 4 protein has a glycine substituted for methionine to eliminate the start site of the 56-kDa gene 4 protein but is indistinguishable from the wild-type protein (21). T7 DNA polymerase was provided by S. Tabor (Harvard Medical School). Escherichia coli DH5α was obtained from GIBCO/BRL, and strain BL21(DE3) was obtained from Novagen. Plasmid pGPl74(G64) has been described (13).

Mutagenesis of T7 Gene 4. Oligonucleotide primers g4R272Bam(5’ GCC CGC GGA TAC-3’) and g4Nde 5’-AGA TAT ACA 5’ were used in the PCR to attach Ndel and BamHI restriction sites and to amplify T7 gene 4 from the plasmid pGP4-G64(5) described. The 950-bp product was purified from an agarose gel, was digested with Ndel and BamHI, was purified, and was ligated into the Ndel and BamHI sites of pET24a plasmid (Novagen). In the resulting plasmid, pET44P, the truncated gene 4 was under control of a T7lac promoter. The plasmid pET44P was used to transform strain DH5α for sequencing and strain BL21(DE3), for protein expression.

Purification of the Primase Fragment. Colonies of strain BL21(DE3) containing pET44P were inoculated into Luria–Bertani medium containing 60 μg/ml Kanamycin. At OD600 of 0.5, isopropyl β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and the cells were grown at 37°C for 3 hr. The induced cells were harvested, washed, and collected by
measured by following the dissociation of 5 as described (23). Products were measured by using a Fuji triphosphates was monitored by using thin-layer chromatography. 

After centrifugation at 15,000 × g for 30 min, the supernatant was designated Fraction I.

Fraction I was diluted to 10 mg/ml, and a 10% solution of streptomycin sulfate was added to a concentration of 1%. After centrifugation at 15,000 × g for 30 min, the supernatant contained the primase fragment (Fraction II).

Fraction II (19 ml) was loaded onto a column (4.9 cm × 50 cm) of DEAE Sephrose and washed with 400 ml of 50 mM Hepes (pH 7.5) and 0.1 mM DTT (Buffer B). The primase fragment was eluted with Buffer B containing a gradient of NaCl from 0 to 1 M. The fractions containing the primase fragment were combined (24 ml), and EDTA was added to a concentration of 1 mM. Ammonium sulfate (12 g) was added with stirring, and the precipitate was collected by centrifugation and dissolved in 5.5 ml of Buffer A (Fraction III).

Fraction IV (5 ml) was loaded onto a Sephacryl S-200HR column (4.9 cm × 10 cm) and was eluted with 50 mM Hepes, 1 mM EDTA, 0.1 mM DTT, and 50 mM NaCl. The fractions containing the primase fragment (38 ml) were combined (Fraction IV). 

PAGE. PAGE was performed with 10% polyacrylamide gels (22) by using a Mini-PROTEAN II Electrophoresis system (Bio-Rad). For denaturing electrophoresis, 0.1% SDS was included in the loading dye and electrophoresis buffer, and samples were boiled for 5 min before loading.

The hydrolysis of nucleoside triphosphates was monitored by using thin-layer chromatography as described (23). Products were measured by using a Fuji BAS1000 Bio-imaging Analyzer.

Helicase Assay. Helicase activity of the T7 gene 4 protein was measured by following the dissociation of [52P]-labeled oligonucleotide annealed to M13 ssDNA. The 36-base oligonucleotide 5′-GGATCCGGGAATTCGTAATCGCCTAAGGCTAAC-3′ was added in equal molar excess. The primer changed the direction of replication and the 3′-terminus of the 63-kDa gene 4 protein. After digestion with nuclease S1, the nicked DNA was incubated with the enzyme and unlabeled nucleotides. The hydrolysis of nucleoside triphophates was monitored by thin-layer chromatography.

Strand Displacement DNA Synthesis. Helicase activity also can be measured by the ability of gene 4 proteins to stimulate the activity of T7 DNA polymerase on duplex templates (24). We have used a mini-circle containing a preformed replication fork (provided by J. Lee, Harvard Medical School) that consists of a 271 bp DNA molecule with a 3′-hydroxyl terminus and a 5′-ssDNA tail. Reactions (20 μl) were performed as described (25, 26) with either 100 mM 63-kDa gene 4 protein or primase fragment. Radioactive nucleotides incorporated into DNA were measured as described (8).

Oligonucleotide Synthesis Assay. Oligonucleotide synthesis was measured as described (27, 28) in reactions containing either 100 mM 63-kDa gene 4 protein or primase fragment. Standard 10-μl reactions contained either M13 ssDNA or a 20-base oligonucleotide 5′-GGTGACCCGAGATCCTACG-3′ with 100 nM of either the 63-kDa gene 4 protein or primase fragment.

Oligonucleotide Extension Assay. Oligonucleotide extension reactions using either 100 mM 63-kDa gene 4 protein or primase fragment were performed as described for the oligo-

RESULTS

A Plasmid Encoding the Primase Fragment. Ilina et al. (17) have shown that there is significant homology between the N-terminal residues of the 63-kDa gene 4 protein and other prokaryotic DNA primases. Based on amino acid alignments, the helicase activity should be contained within the region between residues 272 and 566. Consequently, we have constructed a gene 4 encoding two stop codons after the codon for leucine residue 271. The primase domain was amplified by using the PCR from plasmid pGP4-G64 at 30°C, which lacks the initiation codon for the 56-kDa gene 4 protein. The upstream primer introduced an NdeI site at the 5′-end of gene 4. The downstream primer changed codon 272 to a stop (TGA) codon and introduced a BamHI site at the 3′-end of the truncated gene. After digestion with NdeI and BamHI, the PCR product was ligated into the vector pET24a and was placed under control of a T7 (lac) promoter to create the plasmid pETG4-PF.

Purification and Physical Properties of the Primase Fragment. Over-production of the primase fragment was achieved by induction of E. coli cells harboring plasmid pETG4-PF encoding the primase fragment under the control of the T7 (lac) promoter. After growth and lysis of the induced cells, the primase fragment was purified to apparent homogeneity (Materials and Methods). Purification was followed by analysis of proteins on a denaturing polyacrylamide gel (Fig. 1A). The primase fragment represents 40% of the protein present in the lysate (Fraction I) as shown in Fig. 1A, lane 2. After removal of DNA by precipitation with streptomycin sulfate (Fig. 1A, lane 3), the protein was fractionated by chromatography on DEAE-Sepharose (Fig. 1A, lane 4),

![FIG. 1. SDS and native PAGE of the primase fragment. (A) Proteins were separated on a 10% polyacrylamide gel in the presence of 1% SDS. Lanes: 1, 2 μg each of protein standards; 2, 20 μg of Fraction I, a cleared lysate of BL21(DE3) cells containing the plasmid pETG4-PF that were induced with isopropyl β-D-thiogalactopyranoside for 3 hr; 3, 20 μg of Fraction II, the supernatant after the precipitation of nucleic acids with streptomycin sulfate; 4, 5 μg of Fraction III, the DEAE-Sepharose chromatography pool; 5, 5 μg of Fraction IV, the pool from the Sephacryl S200HR column; 6, 5 μg of Fraction V, the pure primase fragment after chromatography on a Hi-trap Blue affinity column. (B) Proteins were analyzed on a native polyacrylamide gel run under nondenaturing conditions. Lanes: 1, 2 μg of primase fragment (Fraction V); 2, 2 μg of the 63-kDa gene 4 protein. The positions of the same protein standards shown in A are indicated.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1211999/figure/1/)
followed by gel filtration (Fig. 1A, lane 5) and finally by chromatography on Hitrap Blue affinity resin (Fig. 1A, lane 6). Homogeneous protein (~20 mg/l of cell culture) was obtained.

There are several noteworthy differences between the expression and purification of the primase fragment and full-length gene 4 protein. First, whereas the primase fragment comprised 40% of the total protein, the full-length protein is expressed only to a maximum of 5–10% (21, 31). Second, the primase fragment is more soluble in extracts; more than half of the 63-kDa gene 4 protein is lost to inclusion bodies compared with <20% of the primase fragment. Third, the primase fragment does not purify together with DNA. This finding is not surprising because amino acid changes located in the helicase domain decrease the ability of the protein to bind DNA (13, 16). Finally, the primase fragment does not bind to an ATP agarose column, unlike the 63-kDa protein (6). This result is likely caused by the loss of the conserved nucleotide binding site in the helicase domain.

The purified primase fragment appears homogeneous on both a denaturing (Fig. 1A) and a nondenaturing (Fig. 1B) polyacrylamide gel. The primase fragment migrates as a single species with a mass of 60 kDa. Under the same conditions, the 63-kDa protein migrates as two species, a 63-kDa monomer and a 252-kDa hexamer. This result suggests that the primase fragment exists in solution only as a dimer. It was suggested that the gene 4 hexamer is formed from a combination of three dimers because gene 4 proteins containing amino acid substitutions for His475 or Asp485 formed dimers but not hexamers (23).

**Absence of Translocation and Helicase Activities.** Gene 4 protein catalyzes the ssDNA-dependent hydrolysis of dTTP (3, 4, 21, 32). The energy of hydrolysis is used to fuel the 5'-to-3' translocation of the protein (5) and to unwind duplex DNA (2, 33, 34). The primase fragment has no detectable dTTPase activity whereas the full-length protein catalyzes the hydrolysis of dTTP at a rate of 1.4 μmol/min/mg (Fig. 2).

Although the lack of dTTPase ensures an absence of helicase activity, we also have examined helicase activity in two assays. We measured the ability of the primase fragment to displace an oligonucleotide annealed to ssDNA (24). In direct comparison with 63-kDa gene 4 protein, no displacement of the oligonucleotide was observed, even at concentrations 50-fold in excess of that required for the full-length protein (data not shown). In addition, we have measured the helicase activity of the primase fragment indirectly in an assay in which T7 DNA polymerase activity depends on helicase activity to expose ssDNA template on dsDNA. The DNA template was a 70-nt circular oligonucleotide annealed with a linear 110-nt oligonucleotide to form a replication fork (25, 26). In the presence of full-length gene 4 protein, DNA polymerase catalyzed the polymerization of nucleotides on this duplex template at a rate of 20 to 40 pmol/min whereas no detectable (~0.5 pmol/min) synthesis was found with the primase fragment, even at a 100-fold higher protein concentration.

**Template-Directed Oligonucleotide Synthesis.** On a ssDNA template containing a T7 primase recognition site, 5'-GTC-3', the 63-kDa gene 4 protein catalyzes the synthesis of the dinucleotide pppAC from precursors ATP and CTP (28). Although essential for recognition, the cytosine in the recognition sequence is not copied into the primer. T7 primase extends these dinucleotides to tri- and tetranucleotides at the recognition sequences 5'-G(T/G)GTC-3' and 5'-GTGC-3' (5, 35).

Like the full-length protein, the primase fragment catalyzes template-directed synthesis of oligoribonucleotides. In the absence of ssDNA, no synthesis of oligoribonucleotides is detected in assays containing ATP, [α-32P]CTP, and either the 63-kDa protein (Fig. 3, lane 1) or the primase fragment (Fig. 3, lane 2).
When M13 ssDNA is present, both the 63-kDa protein (Fig. 3, lane 3) and the primase fragment (Fig. 3, lane 4) catalyze the synthesis of oligoribonucleotides. In all of the reactions, the same concentration of primase fragment or 63-kDa protein was used based on the mass of a monomer. It is important to note that both proteins synthesized similar amounts and similar lengths of oligonucleotides. By using a synthetic ssDNA template of defined sequence, we can identify these oligonucleotides. On a template containing the recognition sequence 5'-GGGTC-3', both the 63-kDa protein (Fig. 3, lane 5) and the primase fragment (Fig. 3, lane 6) synthesize similar amounts of pppAC, pppACC, and pppACCC.

Although the primase fragment and the full-length protein catalyze the synthesis of oligonucleotides at similar rates, significant differences are apparent when dTTP is added to the reaction mixture. dTTP increases the affinity of the gene 4 protein for ssDNA, and its hydrolysis provides the energy for helicase translocation. Consequently, the primase activity of the 63-kDa gene 4 protein is stimulated greatly by dTTP. Stimulation is greatest when long templates such as M13 DNA are used as templates because translocation increases the frequency with which the protein encounters primase recognition sites (21). To minimize the effect of translocation, we have examined the effect of dTTP on oligonucleotide synthesis by using a 20-base template containing a primase recognition site (Fig. 4). In reactions containing [α-32P]CTP and ATP, the addition of dTTP increased the rate of oligoribonucleotide synthesis by the 63-kDa protein up to 11-fold but had no effect on synthesis catalyzed by the primase fragment. In addition, the average length of the products in the reaction catalyzed by the 63-kDa protein increases on addition of dTTP. The synthesis of tetranucleotides is stimulated >22-fold in the presence of dTTP whereas the synthesis of dimers is increased only 4-fold (Fig. 4, Insert). The ratio of dimers:tetramers synthesized by the primase fragment is not altered by the addition of dTTP.

Because helicase translocation most likely plays a small role in the stimulation of oligoribonucleotide synthesis with short templates, the stimulation by dTTP likely results from an increased affinity for the template. We used a kinetic analysis to examine the affinity of the primase fragment for a 20-nt oligonucleotide template (5'-GGGTCX15-3') in the presence and absence of dTTP. The effect of DNA template concentration on oligonucleotide synthesis is essentially identical for both proteins in the absence of dTTP (Fig. 5). In the absence of dTTP, a double-reciprocal analysis yields a K\text{apparent} for the DNA of 5 ± 4 μM and 10 ± 8 μM for the 63-kDa gene 4 protein and the primase fragment, respectively. We conclude that the deletion of the helicase domain has no effect on the affinity of the primase for the template in the absence of dTTP. The 63-kDa protein is stimulated greatly by the presence of dTTP with a K\text{apparent} for the DNA of 0.4 ± 0.3 μM, indicating a 12-fold increase in affinity.

**Dinucleotide Extension.** Although the primase fragment and the full-length enzyme have nearly identical kinetic properties with regard to oligoribonucleotide synthesis in the absence of dTTP, caution must be used in this comparison. The full-length protein functions as a hexamer whereas the primase fragment is a dimer. Likewise, the NTP hydrolysis site in the intact gene 4 protein, although preferring dTTP, can use ATP hydrolysis for translocation (4). Thus, the omission of dTTP may not eliminate binding of the 63-kDa protein to ssDNA.

In addition to catalyzing the de novo synthesis of oligoribonucleotides from NTPs, T7 DNA primase mediates the annealing of dinucleotides at recognition sites and catalyzes their extension (29). For example, the 63-kDa protein extends 5'-AC-3' bound to the primase recognition site 5'-GGGTCX15-3' on ssDNA to 5'-ACCC-3' in the presence of CTP. By replacing ATP with AC, only CTP is required for extension. Because CTP is not a substrate to fuel helicase translocation (4), oligonucleotide synthesis catalyzed by the primase fragment can be compared more directly to that by the full-length protein.

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**Fig. 4.** Effect of dTTP on oligoribonucleotide synthesis. The products of oligonucleotide synthesis assays containing ATP, [α-32P]CTP, and 10 μM 20-base oligonucleotide (5'-GGGTCX15-3') were separated on 25% polyacrylamide gel containing 2 M urea, and their amounts were measured by using a Fuji BAS1000 Bio-imaging Analyzer. The amounts of di-, tri-, and tetranucleotide synthesized were normalized assuming on labeled nucleotide per dinucleotide (pppAC), two labeled nucleotides per trinucleotide (pppACC), and three labeled nucleotides per tetranucleotide (pppACCC). The rates of oligoribonucleotide synthesis are shown for reactions catalyzed by the 63-kDa gene 4 protein (●) or the primase fragment (○). (Inset) The rates of dinucleotide (white), trinucleotide (gray), and tetranucleotide (black) synthesis by the 63-kDa gene 4 protein or the primase fragment in the presence and absence of 0.6 mM dTTP.

**Fig. 5.** Effect template concentration on oligoribonucleotide synthesis. The products of oligonucleotide synthesis assays containing ATP and [α-32P]CTP were as described in Fig. 4. The rates of oligoribonucleotide synthesis were determined for reactions containing the indicated concentrations of 20-base oligonucleotide (5'-GGGTCX15) and either the primase fragment (○), the 63-kDa gene 4 protein (●), or the 63-kDa gene 4 protein in the presence of 1 mM dTTP (▲).
DISCUSSION

The finding that gene 4 of bacteriophage T7 encodes both a helicase and a primase whereas these activities are usually encoded by separate genes in other organisms (17, 37) suggests that these activities reside in separate domains. Considerable indirect evidence has accumulated to support this hypothesis. First, gene 4 actually encodes two polypeptides, a 56- and a 63-kDa protein, the former arising from an internal translational initiation site (37). The 56-kDa protein has helicase activity but no primase activity (9). The 63-kDa protein is essential for T7 growth whereas the 56-kDa protein is not (18, 19).

Primase activity depends on the cys1 zinc finger located within the 63-aa residues that are unique to the full-length protein. However, the zinc finger itself is not sufficient for primase activity because the 56-kDa protein contains the active site for phosphodiester bond formation (9, 11). The fact that the 56-kDa gene 4 helicase contains the site for phosphodiester bond formation (9) suggests that the intact primase domain consists of the zinc finger plus an N-terminal portion of the 56-kDa protein. In support of this interpretation, electron micrographs of the full-length protein suggest a bilobal shape (38), and a C-terminal fragment has been isolated that has helicase activity (15). The primase fragment described here catalyzes template-directed oligonucleotide synthesis comparable to that of the 63-kDa protein.

The only significant difference between the primase activity of the full-length protein and the primase fragment occurs in the presence of dTTP. The full-length gene 4 protein binds ssDNA and translocates 5′ to 3′ along the DNA concomitant with the hydrolysis of dTTP. The binding of dTTP produces a conformational change in gene 4 protein (39) that affects both DNA binding and translocation. Both the binding and translocation of gene 4 protein can affect oligoribonucleotide synthesis. Binding to ssDNA, mediated through dTTP binding to the nucleoside binding site of the helicase domain, stimulates oligonucleotide synthesis by stabilizing the protein on ssDNA (22). The translocation activity provides an efficient mechanism by which the primase domain can be brought to primase recognition sites (5, 27). The fact that dTTP stimulates oligoribonucleotide synthesis on long DNA templates may be attributed primarily to increased helicase translocation. On oligonucleotides containing a recognition site, the stimulation of the 63-kDa protein by dTTP likely is caused by an increased affinity for ssDNA. Hence, in the presence of dTTP, the primase fragment and full-length protein have comparable activities at high DNA template concentrations whereas the activities differ greatly at low template concentrations.

Another noteworthy difference between the primase fragment and the full-length protein is their subunit composition. Wild-type

![Fig. 6.](image-url)  
*Fig. 6. Extension of dinucleotides. The products of dinucleotide extension assays containing [α-32P]CTP and 10 μM 20-base oligonucleotide (5′-GGGTCX₃₋₅) were analyzed as described in Fig. 4. The relative amounts of tri- and tetranucleotide synthesized were normalized assuming one labeled nucleotide per two labeled nucleotides per trinucleotide (ACC) and three labeled nucleotides per tetranucleotide (ACCC). Rates of dinucleotide extension are reported at various dinucleotide (AC) concentrations for reactions containing the primase fragment (○), the 63-kDa gene 4 protein (●), or the 63-kDa gene 4 protein in the presence of 1 mM dTTP (▲).*

![Fig. 7.](image-url)  
*Fig. 7. Interaction between the primase fragment and T7 DNA polymerase. The reaction mixtures contained 12 μg/ml M13, 100 μM CTP, 100 μM ATP, and 300 μM each of [α-32P]dGTP, dATP, dCTP, and dTTP. After a preincubation at 30°C for 10 min, reactions were initiated by the addition of T7 DNA polymerase premixed with either the primase fragment or the 63-kDa gene 4 protein. Rates of incorporation of nucleotides into DNA are reported for reactions containing various amounts of the primase fragment (○) or the 63-kDa gene 4 protein (●).*
gene 4 protein functions as a hexamer (23, 40) that surrounds the DNA (33, 38). In vitro the maximum helicase and primase activities are achieved only when a mixture of the two forms is present (6, 28, 31). We find that under conditions where the 63-kDa protein forms hexamers, the primer fragment forms dimers exclusively. This finding is not surprising because residue substitutions in the C-terminal helicase domain also disrupt the hexamer, resulting in a dimeric protein (23). These results suggest that the C-terminal helicase domain is required for one of the subunit interfaces in the hexamer and that a distinct subunit–subunit interaction is mediated by the N-terminal primase domain (23). In a similar manner, the dimeric T4 gp41 helicase assembles into hexamers on binding a nucleoside triphosphate (41).

The physical association of the helicase and primase activities results in more efficient primer synthesis. In systems in which the primase and helicase functions reside in separate proteins, the proteins form a complex. In E. coli, the dnaG-encoded DNA primase and the dnaB-encoded helicase form a complex (42), and, in phage T4, the gp41 protein (helicase) combines in a 6:1 complex with the T4 gp61 protein (primase) when bound to DNA and ATP (43–45). It was proposed that the T7 gene 4 evolved for primase function can be isolated independently. The physical association of the helicase and primase activities into a single protein molecule.

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