Analysis of a Multicomponent Thermostable DNA Polymerase III Replicase from an Extreme Thermophile*

Irina Bruck‡, Alexander Yuzhakov§§, Olga Yurieva§, David Jeruzalmi§, Maija Skangalis§§, John Kuriyan§§, and Mike O’Donnell§§§

Received for publication, October 23, 2001, and in revised form, February 18, 2002
Published, JBC Papers in Press, February 21, 2002, DOI 10.1074/jbc.M110198200

From ‡The Rockefeller University and §Howard Hughes Medical Institute, New York, New York 10021

This report takes a proteomic/genomic approach to characterize the DNA polymerase III replication apparatus of the extreme thermophile, Aquifex aeolicus. Genes (dnaX, holA, and holB) encoding the subunits required for clamp loading activity (γ, δ, and δ’) were identified. The dnaX gene produces only the full-length product, α, and therefore differs from Escherichia coli dnaX that produces two proteins (γ and τ). Nonetheless, the A. aeolicus proteins form a σδδ’ complex. The dnaN gene encoding the β clamp was identified, and the σδδ’ complex is active in loading β onto DNA. A. aeolicus contains one dnaE homologue, encoding the α subunit of DNA polymerase III. Like E. coli, A. aeolicus α and τ interact, although the interaction is not as tight as the α–τ contact in E. coli. In addition, the A. aeolicus homologue to dnaQ, encoding the ε proofreading 3’–5’-exonuclease, interacts with α but does not form a stable αε complex, suggesting a need for a brake or bridging protein to tightly couple the polymerase and exonuclease in this system. Despite these differences to the E. coli system, the A. aeolicus proteins function to yield a robust replicase that retains significant activity at 90 °C. Similarities and differences between the A. aeolicus and E. coli pol III systems are discussed, as is application of thermostable pol III to biotechnology.

Chromosomal replicases of all cellular organisms studied thus far are composed of three components, the DNA polymerase, a ring-shaped DNA sliding clamp, and a clamp loader that uses ATP to assemble the sliding clamp onto DNA (1–3). In bacteria, the sliding clamp is a homodimer called β (4). The ring-shaped β dimer completely encircles DNA and slides along the duplex (5). The β clamp also binds the DNA polymerase III, thereby tethering it to DNA for high processivity (5).

This report on the Aquifex aeolicus pol III† replicase is part of our continuing study of comparing and contrasting replicases from a variety of bacteria. Most knowledge of bacterial DNA polymerase III (pol III) structure and function has been obtained from studies of the Escherichia coli replicase, DNA polymerase III holoenzyme (reviewed in Ref. 6). Therefore, a brief overview of its structure and function is instructive for the comparisons to be made in this report. In E. coli, the catalytic subunit of DNA polymerase III is the α subunit (129.9 kDa) encoded by dnaE; it lacks a proofreading exonuclease (7). The proofreading 3’–5’-exonuclease activity is contained in the ε (27.5 kDa) subunit (dnaQ) that forms a 1:1 complex with α (8, 9). The pol III α–ε complex is found tightly associated to a third subunit, called θ, to form the heterotrimeric E. coli DNA polymerase III core (10). The subunit (holE, 8.6 kDa) is not essential for growth and is generally not conserved in bacteria (11).

The E. coli pol III α subunit and pol III core subassembly act distributively on primed ssDNA and have low activity; they are even further inhibited by the presence of SSB (7, 12). However, after the β clamp has been assembled onto a primed site, the efficiency of the pol III α subunit is greatly stimulated, and αψ extends the primer at a rate of ~300 nucleotides/s with a processivity of 1–3 kb (9). The pol III αε complex and pol III core subassembly are even further stimulated by β and extend DNA at a rate of about 1 kb/s with a processivity that exceeds the entire 7.2-kb M13mp18 ssDNA template (9).

The E. coli clamp loader of pol III consists of five different subunits, γ, δ, δ’, χ, and ψ, but only three of them, γ (dnaX, 47.5 kDa), δ (holA, 38.7 kDa), and δ’ (holB, 36.9 kDa), are essential for clamp loading activity in vitro (13). Homologues to E. coli γ (holC, 16.6 kDa) and ψ (holD, 15.2 kDa) subunits can only be identified in a few other organisms so far. The γ and δ’ subunits are homologous to one another and are members of the AAA+ family of proteins (14–16). The δ subunit shows no homology to γ and δ’, but the δβ, δ’ and γδδ’ crystal structures show that δ has the same three domain structure and chain folding pattern as γ and δ’ (17–19). Crystal structure analysis reveals that the five subunits of the γδδ’δ1 complex are arranged as a circular pentamer (19).

Mechanistic studies have outlined the overall mechanism of the clamp loader and are consistent with the structural analysis. The γ subunit is the only subunit that interacts with ATP and therefore is the motor of the clamp loader (20). The δ subunit alone can open one interface of the β dimer (21, 22). The δ clamp opener is sequestered within γ complex via association to δ’ (21). ATP binding to γ results in a conformational change, releasing the β interactive site on δ from δ’ (21, 23). The ATP-γδδ’ species binds to β, opens the ring, and binds DNA (24, 25). Then hydrolysis of ATP brings δ back onto δ’, severing connections to β, allowing β to close around DNA (21, 26–28).

In E. coli, the dnaN gene encoding γ also encodes the τ subunit of DNA pol III holoenzyme (29–31). τ (71.1 kDa) is the...
full-length product of dnaX, whereas γ is shorter (47.5 kDa), being truncated by a translational frameshift. τ can fully replace γ in the clamp loader, and the τδβ′ complex is active in clamp loading (13). The C-terminal sequences unique to τ are required for interaction with the pol III α subunit (32) and also with the replicative DnaB helicase (33, 34). Therefore, within the holoenzyme, τ subunits must replace two (or all three) of the γ subunits in order to connect the two pol III core polymerases in the holoenzyme structure for simultaneous replication of both leading and lagging strands (60).

We have undertaken the study of other bacterial replication systems in an effort to delineate those features of prokaryotic replicases that are general to all bacteria. Study of the Gram-negative Thermus thermophilus dnaX gene showed that it produces both γ and τ, like E. coli dnaX (35, 36). However, instead of a 1 ribosomal frameshift, T. thermophilus employs a transcriptional slippage mechanism that results in both 1 and 2 frameshifts (35, 37). We have also examined the pol III replicase of a Gram-positive organism, Streptococcus pyogenes (38). This study showed that only one protein is produced from the S. pyogenes dnaX gene. This full-length γ protein (62.1 kDa) is intermediate in length between E. coli γ (47.5 kDa) and τ (71.5 kDa) but retains the capacity to bind the DNA polymerase, like E. coli γ. However, the strength of this interaction is weaker than in the E. coli system. Like other Gram-positive bacterias, the S. pyogenes DNA polymerase, pol C (~165 kDa), contains an inherent 3′→5′-exonuclease activity instead of delegating this proofreading action to a separate ε subunit as observed in the E. coli system (39). As in E. coli, the S. pyogenes, τ, δ, and ε subunits are required to load β onto DNA, and the clamp endows pol C with the same rapid speed and processivity as the entire E. coli DNA pol III holoenzyme (38). It is interesting to note that S. pyogenes also contains a second homologue to E. coli α, which we refer to as the DnaE polymerase (38). DnaE polymerase is similar in size to E. coli α (120 kDa) and like E. coli α it lacks 3′→5′-exonuclease activity (38). The processivity of S. pyogenes DnaE polymerase is stimulated by τδ′ and β, but its intrinsic speed (60 nucleotides/s) is unaltered (38).

This report examines the pol III replication machinery of the extreme thermophile, A. aeolicus. By using the known genome sequence (41), we identify A. aeolicus replicase genes and produce and isolate recombinant α, ε, β, τ, δ, ε subunits and SSB. We then compare and contrast the function and assembly of these replicase subunits from a thermophile to the Gram-negative E. coli replicase and to our previous studies on the Gram-positive S. pyogenes replicase.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radioactive nucleotides were from PerkinElmer Life Sciences; unlabeled nucleotides were from Amer sham Biosciences and The Upjohn Co. DNA oligonucleotides were synthesized by Invitrogen. M13mp18 ssDNA was purified from phage that was isolated by two successive bandings in cesium chloride gradients as described (42). M13mp18 ssDNA was primed with a DNA 30-mer (map position 6817–6846) as described (9). The pET protein expression vectors and BL21 (DE3) protein expression strain of E. coli were purchased from Novagen. DNA modification enzymes were from New England Biolabs. A. aeolicus genomic DNA and A. aeolicus cells were a gift of Dr. Robert Huber and Dr. Karl Stetter (Regensburg University, Germany). Protein concentrations were determined using Protein Stain (Bio-Rad) and BSA as a standard. Polyclonal antisera was produced by rabbits injected with purified E. coli γα or α (5). Antibodies directed to A. aeolicus τ were purified from antisera by transfer of E. coli τ from an SDS gel to nitrocellulose by incubation in 0.05 M Tris-HCl, pH 7.5, 0.2 M NaCl, and elution of purified antibody from the nitrocellulose membrane.

**Purification of A. aeolicus α**—The A. aeolicus dnaE gene (41) was amplified from A. aeolicus genomic DNA by PCR using the following primers. The upstream 35-mer (5′-GGTTGTCATATGAGTAAGGATTTGCTGACCTACCTACC-3′) contains an NdeI site (underlined); the downstream 34-mer (5′-GGTGTGATCCGGAGCT-3′) contains a BamHI site (underlined). The PCR product was digested with NdeI and BamHI, purified, and ligated into the PE24 NdeI and BamHI sites to produce pETAdnaE.

The pETAdnaE plasmid was transformed into the BL21 (DE3) strain of E. coli. Cells were grown in 50 liters of LB containing 100 μg/ml kanamycin, 5 mg MgSO4 at 37 °C to an A600 of 2.0, and then supplemented with 2 mM IPTG for 20 h at 20 °C, and then collected by centrifugation. Cell fractions were resuspended in 400 ml of 50 mM Tris-HCl (pH 7.5), 10% sucrose, 1 M NaCl, 30 mM spermidine, 5 mM DTT, and 2 mM EDTA. The following procedures were performed at 4 °C. Cells were lysed by passing them twice through a French press (15,000 pounds/square inch) followed by centrifugation at 13,000 rpm at 4 °C. In this protein preparation, as well as each of those that follow, the induced A. aeolicus protein was easily discernible as a large band in an SDS-polyacrylamide gel stained with Coomassie Blue. Hence, column fractions were assayed for the presence of the A. aeolicus protein by SDS-PAGE analysis, which forms the basis for pooling column fractions.

The clarified cell lysate was heated to 65 °C for 30 min, and the precipitate was removed by centrifugation at 13,000 rpm in a GS rotor for 1 h. The supernatant (1.4 g, 280 ml) was dialyzed against buffer A (20 mM Tris-HCl (pH 7.5), 10% glycerol, 0.5 mM EDTA, 5 mM DTT) overnight, and then diluted to 320 ml with buffer A to a conductivity equal to 100 mM NaCl. The dialysate was applied to a 150-ml fast flow Q-Sepharose column (Amersham Biosciences) equilibrated in buffer A, followed by a 1.5-ml linear gradient of 0–1.0 M NaCl in buffer A. Eighty fractions were collected. Fractions 38–58 (1 g, 390 ml) were pooled, dialyzed versus buffer A overnight, and applied to a 250-ml heparin-agarose column (Bio-Rad) equilibrated with buffer A. Protein was eluted with a 1-liter linear 0–500 mM NaCl gradient in buffer A. One hundred fractions were collected. Fractions 69–79 (320 mg in 200 ml) were pooled and dialyzed against buffer A containing 100 mM NaCl. The α preparation was aliquoted and stored frozen at −80 °C. The Coomassie Blue-stained SDS-polyacrylamide gel of the final α preparation is shown in Fig. 1.

**Purification of ε Encoded by dnaQ**—The A. aeolicus dnaQ gene was identified in the genome sequence as a 202-residue protein of 17,132 Da having significant homology to E. coli dnaQ (41). A. aeolicus dnaQ was amplified by PCR using the following oligonucleotide primers. The upstream 36-mer (5′-GGTGGTCATATGAGTAAGGATTTGCTGACCTACCTACC-3′) contains an NdeI site (underlined), and the downstream 44-mer (5′-GGTGATCCGGAGCTAAGGATTTGCTGACCTACCTACC-3′) contains a BamHI site (underlined). The PCR product was digested with NdeI and BamHI, purified, and ligated into the PE24a vector to produce pETAdnaQ.

The pETAdnaQ plasmid was transformed into E. coli strain BL21 (DE3). A single colony was used to inoculate 12 liters of LB media supplemented with 200 μg/ml ampicillin. Cells were grown at 37 °C to an A600 = 0.5 at which point 0.5 mM IPTG was added. After a 3-h induction, cells were collected by centrifugation and resuspended in 50 ml Tris-HCl (pH 7.5), 10% sucrose, 1 M NaCl, 30 mM spermidine, 5 mM DTT, 2 mM EDTA. Cells were lysed by two passages through a French press (15,000 pounds/square inch) followed by centrifugation at 13,000 rpm at 4 °C. The solution was clarified by centrifugation at 13,000 rpm for 30 min at 4 °C. The resulting supernatant (1038 mg) was incubated in a 65 °C waterbath for 30 min. The solution was clarified by centrifugation at 13,000 rpm for 30 min. The supernatant (426 mg) was dialyzed against buffer A and loaded onto a 15-ml fast flow Q-Sepharose column equilibrated with buffer A. The column was eluted with a 150-ml linear gradient of 50–500 mM NaCl in buffer A; 80 fractions were collected. Peak fractions (fractions 36–44, 12.9 mg) were pooled, dialyzed against buffer A, and then loaded onto a 10-ml heparin-agarose column equilibrated with buffer A. The column was eluted with a linear gradient of 0 mM to 1 mM NaCl; 50 fractions were collected. Peak fractions (fractions 60–70) were pooled, aliquoted, and stored at −80 °C.

**Identification of A. aeolicus holA and Purification of δ**—The A. aeolicus holA gene was not identified previously by the genome sequencing project. We identified A. aeolicus holA by searching the A. aeolicus genome with the amino acid sequence of the E. coli δ subunit (encoded by holA). Although the resulting match had too low a score to be confident of its assignment as δ, the studies of this report prove that the gene truly encodes δ subunit of the replicase. The A. aeolicus holA gene was amplified by PCR using the following primers. The upstream 38-mer (5′-GGTGTCATATGAGTAAGGATTTGCTGACCTACCTACC-3′) contains an NdeI site (underlined); the downstream 39-mer (5′-GGTGATCCGGAGCTAAGGATTTGCTGACCTACCTACC-3′) contains a BamHI site (underlined). The PCR product was digested with NdeI and BamHI, purified, and ligated into the pET24 NdeI and BamHI sites to produce pETAdholA.
The pETAaholA plasmid was transformed into *E. coli* strain BL21 (DE3). Cells were grown in 50 liters of LB media containing 100 μg/ml kanamycin. Cells were grown at 37 °C to an *A*₆₀₀ of 2.0, then induced for 20 h upon addition of 2 mM IPTG, and collected by centrifugation. Cells from 25 liters of culture were lysed as described above for purification of *A. aeolicus*.

The cell lysate was heated to 65 °C for 30 min, and the precipitate was removed by centrifugation. The supernatant (2.4 g, 400 ml) was dialyzed against buffer A and then applied to a 220-ml fast flow Q-Sepharose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–500 mM NaCl in buffer A; 80 fractions were collected. Fractions 23–30 were pooled and diluted 2-fold with buffer A to a conductivity equal to 100 mM NaCl and then loaded onto a 200-ml heparin-agarose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–1.0 M NaCl in buffer A; 84 fractions were collected. Fractions 46–66 were pooled (1.3 g, 395 ml), dialyzed versus buffer A containing 100 mM NaCl, then aliquoted, and stored frozen at –80 °C.

**Identification of A. aeolicus holB and Purification of**

The pETAaholA plasmid was transformed into *E. coli* strain BL21 (DE3). Cells were grown at 37 °C in 50 liters of media containing 100 μg/ml kanamycin to an *A*₆₀₀ of 2.0 and then induced for 3 h upon addition of 2 mM IPTG. Cells were collected by centrifugation and were lysed using lysozyme by the heat lysis procedure. The cell lysate was heated to 65 °C for 30 min, and precipitate was removed by centrifugation. The supernatant (2.4 g, 400 ml) was dialyzed versus buffer A and then applied to a 220-ml fast flow Q-Sepharose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–500 mM NaCl in buffer A; 80 fractions were collected. Fractions 23–30 were pooled and diluted 2-fold with buffer A to a conductivity equal to 100 mM NaCl and then loaded onto a 200-ml heparin-agarose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–1.0 M NaCl in buffer A; 84 fractions were collected. Fractions 46–66 were pooled (1.3 g, 395 ml), dialyzed versus buffer A containing 100 mM NaCl, then aliquoted, and stored frozen at –80 °C.

**Purification of**

---

**Pol III Holoenzyme of Aquifex aeolicus**

**A) SDS-PAGE**

**FIG. 1.** Protein subunits of *A. aeolicus* pol III holoenzyme system. A, subunit preparations were analyzed in a 15% SDS-polyacrylamide gel stained with Coomassie Blue. Proteins were prepared as described under "Experimental Procedures." The positions of protein standards analyzed in the same gel are indicated to the left. B, the table gives the subunit gene name, mass predicted from the gene sequence for *A. aeolicus* pol III subunits, and their percent identity to corresponding subunits in the *E. coli* pol III systems (aligned using ClustalX). The function of subunits, or combinations of subunits, are given in the column at the right.

| gene | Pol III subunit | A. aeolicus mass (Da) | %identity to *E. coli* | function |
|------|----------------|----------------------|------------------------|----------|
| dnaE | α              | 133,207              | 40.7                   | Polymerase |
| dnaN | β              | 41,300               | 24.3                   | Clamp |
| dnaX | τ              | 54,332               | 40.2                   | Clamp Loader |
| holA | δ              | 40,630               | 18.7                   | DNA binding |
| holB | δ'             | 34,896               | 18.6                   |          |
| ssb  | SSB            | 17,132               | 33.8                   |          |
| dnaQ | ε              | 23,044               | 24.8                   | 3'–5' exonuclease |

---

**B) SDS-PAGE**

**FIG. 1.** Protein subunits of *A. aeolicus* pol III holoenzyme system. A, subunit preparations were analyzed in a 15% SDS-polyacrylamide gel stained with Coomassie Blue. Proteins were prepared as described under "Experimental Procedures." The positions of protein standards analyzed in the same gel are indicated to the left. B, the table gives the subunit gene name, mass predicted from the gene sequence for *A. aeolicus* pol III subunits, and their percent identity to corresponding subunits in the *E. coli* pol III systems (aligned using ClustalX). The function of subunits, or combinations of subunits, are given in the column at the right.

The pETAaholA plasmid was transformed into *E. coli* strain BL21 (DE3). Cells were grown at 37 °C in 50 liters of media containing 100 μg/ml kanamycin to an *A*₆₀₀ of 2.0 and then induced for 3 h upon addition of 2 mM IPTG. Cells were collected by centrifugation and were lysed using lysozyme by the heat lysis procedure. The cell lysate was heated to 65 °C for 30 min, and precipitate was removed by centrifugation. The supernatant (2.4 g, 400 ml) was dialyzed versus buffer A and then applied to a 220-ml fast flow Q-Sepharose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–500 mM NaCl in buffer A; 80 fractions were collected. Fractions 23–30 were pooled and diluted 2-fold with buffer A to a conductivity equal to 100 mM NaCl and then loaded onto a 200-ml heparin-agarose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–1.0 M NaCl in buffer A; 84 fractions were collected. Fractions 46–66 were pooled (1.3 g, 395 ml), dialyzed versus buffer A containing 100 mM NaCl, then aliquoted, and stored frozen at –80 °C.

**Purification of**

---

**Encoded by dnaX**—The *A. aeolicus* dnaX gene was amplified by PCR from genomic DNA using the following primers. The upstream 41-mer (5'-GTGTGTGACATATGAATACGTTCCCGAGGAAAGTAGACAG-3') contains an *NdeI* site (underlined); the downstream 35-mer (5'-GTGTGTGAGTCCTAAATTGGCGCTGCAAACCGTGG-3') contains a *BamHI* site (underlined). The PCR product was digested with *NdeI* and *BamHI*, purified, and ligated into the pET24 *NdeI* and *BamHI* site to produce pETAaholB.

The pETAaholB plasmid was transformed into *E. coli* strain BL21 (DE3). Cells were grown at 37 °C in 50 liters of media containing 100 μg/ml kanamycin to an *A*₆₀₀ of 2.0 and then induced for 3 h upon addition of 2 mM IPTG. Cells were collected by centrifugation and were lysed using lysozyme by the heat lysis procedure. The cell lysate was heated to 65 °C for 30 min, and precipitate was removed by centrifugation. The supernatant (2.4 g, 400 ml) was dialyzed versus buffer A and then applied to a 220-ml fast flow Q-Sepharose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–500 mM NaCl in buffer A; 80 fractions were collected. Fractions 23–30 were pooled and diluted 2-fold with buffer A to a conductivity equal to 100 mM NaCl and then loaded onto a 200-ml heparin-agarose column equilibrated in buffer A. Protein was eluted with a 1-liter linear gradient of 0–1.0 M NaCl in buffer A; 84 fractions were collected. Fractions 46–66 were pooled (1.3 g, 395 ml), dialyzed versus buffer A containing 100 mM NaCl, then aliquoted, and stored frozen at –80 °C.
with NdeI and BamHI, purified, and ligated into the pET24 NdeI and BamHI sites to produce pETAadnaN.

The pETAadnaN plasmid was transformed into E. coli strain BL21 (DE3). Cells were grown in 1 liter of LB containing 100 mg/ml kanamycin at 37 °C to an OD600 of 1.0 and then induced for 6 h upon addition of 2 mM IPTG. Cells were harvested by centrifugation and lysed as described for purification of α. The clarified cell lysate was heated to 65 °C for 30 min, and the protein precipitate was removed by centrifugation. The supernatant (1.1 g in 340 ml) was treated with 0.228 g/ml ammonium sulfite followed by centrifugation. The α subunit remained in the pellet which was dissolved in buffer B (20 mM Hepes (pH 7.5), 5 mM EDTA, 2 mM DTT, 10% glycerol) and buffer B to a conductivity equal to 87 mM NaCl. The dialysate (1073 mg, 570 ml) was applied to a 200-ml fast flow Q-Sepharose column equilibrated in buffer A. The column was eluted with a 300-ml linear gradient of 0–500 mM NaCl in buffer A; 32 fractions were collected. Fractions 15–18 (187 mg, 110 ml) were dialyzed versus buffer A, then aliquoted, and stored at −80 °C.

Purification of β encoded by dnaN—The A. aeolicus dnaN gene (41) was amplified from genomic DNA by PCR using the following primers. The upstream 5'-GTGCTGATCATCAAGGTGAAGGTTTACC-3' contains a NdeI site (underlined); the downstream 35-mer 5'-GAGGACCTCGAGTCATGGCTACACCCTCATCGGCAT-3' contains an XhoI site (underlined). The PCR product was digested with NdeI and BamHI, purified, and ligated into the pET24 NdeI and BamHI sites to produce pETAadnaN.

The pETAadnaN plasmid was transformed into E. coli strain BL21 (DE3). Cells were grown in 50 liters of LB containing 100 mg/ml kanamycin at 37 °C to an OD600 of 0.6 and then induced for 20 h at 20 °C upon addition of 10 mM IPTG. Cells were harvested by centrifugation and lysed as described for purification of α. The clarified cell lysate was heated to 65 °C for 30 min, and the protein precipitate was removed by centrifugation. The supernatant (1.1 g in 340 ml) was treated with 0.228 g/ml ammonium sulfite followed by centrifugation. The α subunit remained in the pellet which was dissolved in buffer B (20 mM Hepes (pH 7.5), 5 mM EDTA, 2 mM DTT, 10% glycerol) and buffer B to a conductivity equal to 87 mM NaCl. The dialysate (1073 mg, 570 ml) was applied to a 200-ml fast flow Q-Sepharose column equilibrated in buffer A. The column was eluted with a 300-ml linear gradient of 0–500 mM NaCl in buffer A; 32 fractions were collected. Fractions 15–18 (187 mg, 110 ml) were dialyzed versus buffer A, then aliquoted, and stored at −80 °C.

Purification of δ Complex—The 5'-GTGCTGATCATCAAGGTGAAGGTTTACC-3' contains a NdeI site (underlined); the downstream 35-mer 5'-GAGGACCTCGAGTCATGGCTACACCCTCATCGGCAT-3' contains an XhoI site (underlined). The PCR product was digested with NdeI and BamHI, purified, and ligated into the pET24 NdeI and BamHI sites to produce pETAadnaN.

The pETAadnaN plasmid was transformed into E. coli strain BL21 (DE3). Cells were grown in 1 liter of LB containing 100 mg/ml kanamycin at 37 °C to an OD600 of 1.0 and then induced for 6 h upon addition of 2 mM IPTG. Cells were collected (7 g) and lysed as described in the earlier). Known amounts of pure recombinant A. aeolicus δ were analyzed on a standard gel for use as a standard curve to quantitate the α and β subunits of A. aeolicus cells observed in the Western blot. The protein concentration of A. aeolicus δ was calculated from its absorbance at 280 nm in 6 mM guanidine hydrochloride using the molar extinction coefficient determined from its 2 Trp, 14 Tyr, and 20 Phe content (ε280 = 5,690 × 2 + 1,280 × 14 + 20 = 29,340 cm−1 M−1).

Assays of α, β, δ—Titration of α into replication reactions were performed using circular M13mp18 ssDNA primed with a 700-fold molar excess of synthetic DNA 20-mer oligonucleotide. Reactions contained 138 fmol of primed M13mp18 ssDNA, 100 pmol of DNA 30-mer, 3.6 μg of SSB (when present), 150 ng of α, 0.4 μg of β (when present), and the indicated amount of δ in 50 μl of 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH4)2SO4, 2 mM MgSO4, 0.1% Triton X-100, 4 mM CaCl2, 0.5 mM ATP, and 60 μM each dATP and dGTP. Reactions were mixed on ice, and then the mixture was brought to 65 °C for 2 min before initiating synthesis upon addition of 2 μl of dCTP and [α-32P]dTTTP (final concentrations, 60 and 40 μM, respectively). Aliquots were removed and quenched at the times indicated upon adding EDTA and SDS to final concentrations of 20 mM and 0.5%, respectively. Quenched reactions were then analyzed in a 0.8% alkaline-agarose gel. Gel images were also included in the same gel. Gels were dried following analysis using a PhosphorImager.

Replication time course reactions contained 70 ng (25 fmol) of M13mp18 ssDNA, 100 pmol of 30-mer oligonucleotide, 2 μg of SSB (when present), 100 ng of α, 200 ng of δ complex, and 40 ng of β in 25 μl of 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH4)2SO4, 10 mM MgSO4, 0.1% Triton X-100, 4 mM CaCl2, 10% glycerol, and 0.6% (v/v) E. coli antibody raised against A. aeolicus. Reactions were incubated for 0.6 and then induced at 20 °C and 0 °C, respectively. Quenched reactions were then analyzed in 0.8% alkaline-agarose gels. Size standards were included in the same gel. Gels were dried and exposed to film.

Detection of a Interaction with τ and by Elisa—The α−τ interaction was analyzed as follows: E. coli α or A. aeolicus α (2 μg in 100 μl) were placed in wells of a 96-well vinyl assay plate (Costar) and incubated for 12 h at 4 °C in 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2 mM DTT, 5 mM MgCl2, 100 mM NaCl, 20% glycerol. Solutions were removed, and wells were washed 4 times with 100 μl of TBS. Wells were then blocked with 100 μl of TBS containing 5% non-fat milk for 3 h at 23 °C. Following the blocking step, wells were washed 4 times with TBS and then incubated with 4 μg of either E. coli τ or A. aeolicus τ for 2 h at 23 °C. Solutions were then removed, and wells were washed as above and then incubated overnight at 4 °C with 100 μl of 1:500 dilution of polyclonal rabbit antibody raised against A. aeolicus δ complex (1 cm) and then incubated with 100 μl of anti-rabbit horseradish peroxidase-conjugated antibody (Sigma) for 30 min and developed with an ECL detection kit (Amer sham Biosciences) as described by the manufacturer. Detection of a and α interaction was performed as described above with the following modifications. One hundred microliters of each
coli or A. aeolicus ε (50 μg/ml) were incubated in wells for 12 h at 4 °C. Following the block step and the washes with TBS, 100 μl of either E. coli α or A. aeolicus α (100 μg/ml) were plated on 10% glycerol; 0.01% Triton X-100, 0.05% Tween 20, 0.01% Nonidet P-40. Wells were then washed with TBS (3 times for 5 min) and further incubated with a 1:10,000 dilution of anti-rabbit horseradish peroxi-
dase-conjugated antibody (Sigma) for 30 min and then developed with an ECL detection kit (Amersham Biosciences) and exposed to film.

Detection of α-β Interaction by DNA Synthesis—DNA synthetic reactions were performed in the presence of M13mp18 ssDNA using a synthetic DNA 60-mer, 1 μg of E. coli SSB, 100 ng of polymerase subunit (either S. pyogenes pol C or E. coli α), and when present 400 ng of τ (S. pyogenes τ or E. coli τ), in 25 μl of 20 mM Tris-HCl (pH 7.5), 4% glycerol, 0.1 mM EDTA, 5 mM DTT, 2 mM ATP, 8 mM MgCl2, 40 μg/ml BSA, and 60 μM each of dGTP and dATP. Reactions were preincubated at 37 °C for 2 min, and then synthesis was initiated upon addition of 1.5 μl of DATP and α-32P-γ-ATP (final concentrations of 60 and 20 μM, respectively). Reactions were allowed to proceed for 5 min prior to being quenched with an equal volume (25 μl) of 1% SDS, 40 μM EDTA. One-half of the quenched reaction was analyzed for total DNA synthesis using DES1 paper as described (43).

Assays of A. aeolicus α and τ utilized a different temperature and concentration. Initial concentrations of DATP and α-32P-γ-ATP (final concentrations of 60 and 40 μM, respectively). Reactions were allowed to proceed for 5 min then being quenched with an equal volume (25 μl) of 1% SDS, 40 μM EDTA. One-half of the quenched reaction was analyzed for total DNA synthesis using DES1 filter paper (43).

Thermostability Assays—A. aeolicus α, β, β+β+, SSB, and α + β+β+ were tested for stability at different temperatures by incubating the proteins at the temperatures indicated and then testing them for activity in the M13mp18 replication assay. Heat treatment was performed in 0.4 ml tubes under mineral oil in 5 μl of 20 mM Tris-HCl (pH 7.5), 5 mM DTT, 5 mM EDTA, and either (a) 0.352 μg of α; (b) 0.2 μg of β; or (c) 1.25 μg of β. The samples were incubated at 70 °C for 10 min or 200 °C for 5 min. After preincubation, the samples were mixed in a volume of 200 μl of buffer containing 300 mM NaCl and analyzed by gel filtration as described above. The controls included α alone (1 mg, 37.5 μM), a mixture of 1 μg of α and 200 μg of ε or 0.5 μg of τ in a volume of 200 μl of buffer A containing 300 mM NaCl. Proteins were incubated at 24 °C for 15 min before injecting the mixture onto an HR 10/30 Superose 12 column equilibrated with buffer A containing 200 mM NaCl and 4% glycerol. A. aeolicus α, and 400 ng of A. aeolicus τ in 25 μl of 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH4)2SO4, 10 mM MgSO4, 0.1% Triton X-100, 1 mM ATP, 4 mM CaCl2, 10% glycerol, 60 μM each of dGTP and dATP. The reaction mixture was incubated at 60 °C for 2 min before initiating synthesis by the addition of 1.5 μl of dCTP and [α-32P]ATP (final concentrations of 60 and 20 μM, respectively). Reactions were allowed to proceed for 5 min before being quenched with an equal volume (25 μl) of 1% SDS, 40 μM EDTA. One-half of the quenched reaction was analyzed for total DNA synthesis using DES1 filter paper (43).

RESULTS

The A. aeolicus Clamp Loader—Studies in the E. coli system demonstrated the need for five proteins to form a processive polymerase III: α, τ (or γ), δ’, δ, and β (9, 13, 46). A sixth protein, the ε-δ’-exonuclease, further enhanced the speed and processivity of the holoenzyme constituted from these five proteins (9). The report on the A. aeolicus genome sequence (41) documented the presence of A. aeolicus dnaE (α), dnaX (γ), dnaN (β), and dnaQ (ε), but holA and holB genes encoding δ and δ’ were not identified. As we have reported previously, δ is a very poorly conserved subunit of the pol III replicase (38). We identified S. pyogenes holA by isolating a gene encoding a weak homologue to E. coli δ and then showing that the putative δ formed an active clamp loader complex with other S. pyogenes subunits (38). In the experiments below, we follow a similar strategy to identify genes encoding both A. aeolicus δ and δ’. A search of the A. aeolicus genome for the A. aeolicus holA gene encoding δ, using E. coli δ subunit as the query, produced very weak matches, the best having about 18% identity to E. coli δ (see Fig. 2A). Likewise, a search for the A. aeolicus holB gene encoding δ’, using E. coli δ’ as query, produced a similarly weak match (Fig. 2B). To determine whether the putative A. aeolicus holA and holB genes truly encode the δ and δ’ subunits of the replicase, we expressed and purified the encoded proteins and then tested them for clamp loading activity with the A. aeolicus dnaX product. The genes encoding the putative A. aeolicus δ and δ’ subunits were cloned into the T7-based pET11g expression plasmid. Purification of A. aeolicus δ and δ’ were performed as described under “Experimental Procedures,” and a sample of each preparation is shown in the SDS-polyacrylamide gel of Fig. 1A.

To test ability of the putative A. aeolicus δ and δ’ subunits to form a clamp loader complex with A. aeolicus α and α (encoded by dnx), we needed to express and purify the product of the A. aeolicus dnaX gene. A. aeolicus dnaX lacks a ribosomal frame

shift sequence, and it also lacks the six or more contiguous A…s in the shift sequence, and it also lacks the six or more contiguous A…s.
Fig. 2. The sequences of A. aeolicus δ and δ′ subunits. The amino acid sequences of A. aeolicus and E. coli δ (A) and δ′ (B) are aligned using ClustalX. The asterisks below the sequence indicate amino acids that are identical between the two sequences.

A) δ

A. aeolicus δ

E. coli δ

... -MTEPIFGQYTYLKLKPDNVEKPVYK- ... 57 (56)

A. aeolicus δ

E. coli δ

... -ECPYPGFKSVKGLQKQDFFFYKQGQK- ... 115 (114)

A. aeolicus δ

E. coli δ

... -DAKQLQIGLQSSSEAYQVFLLQTVTKNLWAGLVAWAEQH- ... 172 (175)

A. aeolicus δ

E. coli δ

... -QIFGNLKQVYVQLGLQKQDFFFYYKQGQK- ... 233 (233)

A. aeolicus δ

E. coli δ

... -LTVLSLQISPQGLKVLQIAAYQVFLLQTVKNLWAGLVAWAEQH- ... 293 (290)

A. aeolicus δ

E. coli δ

... -SVEKKNKSEIAGQKQDFFFYYKQGQK- ... 350 (348)

B) δ′

A. aeolicus δ′

E. coli δ′

... -MEVYPLEKQQTTLHFLQG2 ... 49 (57)

A. aeolicus δ′

E. coli δ′

... -CPCCFCQWYKLEQAYQVFLLQTVKNLWAGLVAWAEQH- ... 102 (117)

A. aeolicus δ′

E. coli δ′

... -CHTIKIEQIKYVKNVYVQLGLQKQDFFFYKQGQK- ... 156 (167)

A. aeolicus δ′

E. coli δ′

... -LLNVRSEAIFFT-LRR- ... 222 (228)

A. aeolicus δ′

E. coli δ′

... -ILMEDQQLNKLQVYKGLQKQDFFFYKQGQK- ... 267 (294)

A. aeolicus δ′

E. coli δ′

... -EKCDTTLYTVLCTT-LFQGKLQVYKMLQPKVQV- ... 305 (303)

report, examination of A. aeolicus cell extracts revealed the presence of only full-length τ subunit, a truncated version (i.e. γ) was not detected. The A. aeolicus τ subunit was purified as described under "Experimental Procedures," and a sample of the protein preparation is shown in Fig. 1A.

In the E. coli system, τ (or γ) forms a heterotrimer with δ and δ′ (13). Even though the crystal structure of E. coli γδδ′ shows that γ contacts both δ and δ′ directly, mixtures of γ (or δ) with either δ or δ′ do not yield either heterodimer (i.e. τδ or τδ′) complex (13). To examine the A. aeolicus τ, δ, and δ′ subunits for protein interactions, combinations of these subunits were mixed and then analyzed by gel filtration on a Superose 12 column. The analysis in Fig. 3A demonstrates that a mixture of τ + δ′ does not yield a heterodimer complex, instead the two proteins elute at distinct positions. This result is similar to observations in the E. coli system (13). Analysis of the τ + δ mixture, in Fig. 3B, shows that the bulk of the τ and δ does not comigrate, although a slight amount of δ appears in the vicinity of τ indicating a weak interaction between them. The elution position of the free δ and δ′ subunits in Fig. 3, A and B, compared with molecular mass standards, indicates that δ and δ′ are each monomeric, similar to δ and δ′ of E. coli and S. pyogenes (13, 38). The τ subunit elutes as an oligomer, possibly a trimer or tetramer, like E. coli τ and S. pyogenes τ (38, 47).

A mix of all three subunits results in their comigration as a τδδ′ complex that elutes earlier than either τ, δ′, or δ′ subunits alone (Fig. 3C). We presume on the basis of E. coli structural studies of γδδ′ that the stoichiometry of A. aeolicus τδδ′ is τδ δ′1 (235.8 kDa) (19, 47). Consistent with this, densitometric scans of SDS-polyacylamide gels of Superose 12 column fractions of τδδ′, constituted using excess δδ′, give a stoichiometry of τδ δ′1 δ′2. Previous study of the E. coli and S. pyogenes pol III systems demonstrated that the δ and δ′ subunits form a 1:1 δδ′ complex (13, 38). Fig. 3D shows that A. aeolicus δ and δ′ also form a δδ′ complex. Analysis of a mixture of δ and δ′ demonstrates that the two subunits coelute in earlier fractions than the elution position of either δ or δ′ alone (compare D with A and B) and thus form a δδ′ complex. Densitometric scans of the gel in Fig. 3D yield a stoichiometry of δ′1 δ′2 (a similar stoichiometry is obtained from an analysis using a 2-fold molar excess of δ′ over δ and scanning the fractions containing δδ′ complex).

Is the A. aeolicus τδδ′ complex active as a clamp loader? To test this, a six-residue kinase recognition site was placed onto DNA, some of the 32P-β should comigrate with the DNA in the excluded volume and resolve from the free 32P-β in the included fractions. The results of this
32P-β clamp loading experiment are shown in Fig. 4; about half of the 32P-β is observed to comigrate with DNA in the excluded fractions (fractions 10–15). As a control, a similar experiment was performed in the absence of A. aeolicus complex. The result demonstrates that essentially no 32P-β is assembled onto the DNA in the absence of A. aeolicus complex. Hence, the A. aeolicus is capable of assembling A. aeolicus clamp onto DNA. The temperature resistance of the A. aeolicus clamp will be demonstrated later in this report, along with the DNA polymerase.

**DnaX Produces Only τ in A. aeolicus**—The experiments of Fig. 5 address whether A. aeolicus cells produce a truncated product (i.e., γ) or whether only full-length protein (τ) is produced. In Fig. 5A A. aeolicus whole cells were analyzed by Western using a rabbit polyclonal antibody raised against E. coli γ which cross-reacts with A. aeolicus τ. The A. aeolicus τ subunit is clearly visible in the analysis. The limit of detection in this assay (e.g., due to background) is such that if a lower molecular weight γ-like product is present then it is present at less than 10% the intracellular level of τ. We have performed this Western several times and have also used even more cells, with results similar to those in Fig. 5. The inability to detect γ in A. aeolicus is consistent with the absence of recognizable signals for transcriptional slippage or a translational frameshift in the A. aeolicus dnaX gene sequence.

To determine the average number of molecules of τ present in...
an A. aeolicus cell, we directly counted the number of A. aeolicus cells in the cell suspension using a microscope. This provided us the number of cells that were analyzed in each lane of the Western in Fig. 5A. The amount of τ detected in cells by Western analysis was then quantitated by scanning the gel with a laser densitometer. The band intensity was converted to the amount of protein in Fig. 5A, lane 1 induced cells. A sample of the protein preparation is shown in the SDS-polyacrylamide gel of Fig. 4.

The A. aeolicus DNA Polymerase—The A. aeolicus α subunit homologue has 40.7% identity to E. coli α. Cloning of the A. aeolicus dnaQ gene into pET11 provided a high level of expression from which 320 mg of α were obtained from 50 liters of induced cells. A sample of the protein preparation is shown in lane 1 of Fig. 1.

Like E. coli α, the A. aeolicus α homologue lacks a region of homology to the E. coli ε subunit exonuclease. Thus, it may be presumed that A. aeolicus α binds to another protein that supplies the proofreading exonuclease function, similar to interaction of E. coli α to the ε 3′–5′-exonuclease. The A. aeolicus genome contains a dnaQ gene encoding a homologue to E. coli ε (24.8% identity) (41). The A. aeolicus dnaQ gene was cloned into pET11 for expression and purification. A sample of the purified A. aeolicus ε is shown in the SDS-polyacrylamide gel of Fig. 1.

In Fig. 6A, A. aeolicus α–ε interaction was analyzed in an Elisa type assay in which ε is adhered to wells of a 96-well plate, followed by blocking additional sites with nonspecific protein. Following this, A. aeolicus α is added, and then unbound α is washed away. Rabbit antibody directed against E. coli α cross-reacts with A. aeolicus α and was used to detect whether A. aeolicus α had bound to ε. The results demonstrate a high signal of α bound to the well that was treated with ε. No α is detected if the well is not pretreated with A. aeolicus ε.

Similar results were obtained using E. coli α and ε (also shown in Fig. 6A). To determine whether A. aeolicus α and ε form a stable complex, we analyzed a mixture of α and ε (37 μM α and 44 μM ε) by gel filtration on an FPLC Superose 12 column; however, the α and ε subunits do not comigrate (Fig. 6D). This result indicates that the α and ε subunits do not tightly interact under these conditions. This experiment has been repeated at a protein concentration of 150 μM α and 90 μM ε, and in the presence of either τ, τδ, or τδ + β, all with negative results (not shown).

A chromosomal replicase is generally expected to contain the 3′–5′-exonuclease activity in tight association with the DNA polymerase activity. The fact that we only detect weak interaction rather than a tight gel filterable complex between A. aeolicus α and ε may be due to any of several factors. For example, α and ε may interact tightly at high temperature. Another possibility is that a tight interaction between α and ε may occur in the presence of primed DNA. It is also possible that other proteins beyond those studied in this report are needed to act as a bridge between α and ε. Yet another explanation may be that recombinant A. aeolicus α or ε is not properly folded (addressed below).

As one measure that the A. aeolicus α and ε subunits preparations are correctly folded, we examined them for catalytic activity. The A. aeolicus α was active on gapped calf thymus DNA at 37 °C but became more active at 65 °C (Fig. 6B). By comparison, E. coli α was more active than A. aeolicus α at 37 °C but was essentially inactive at 65 °C. A. aeolicus α retained significant activity even at 85 °C (about 40% relative to 65 °C). For exonuclease activity, a 5′-32P-end-labeled DNA 50-mer was used to determine whether the A. aeolicus ε subunit was active. The results, in Fig. 6C, show an increase in mobility of the 5′ 32P-DNA oligonucleotide upon incubation with A. aeolicus ε, indicating the presence of 3′–5′-exonuclease activity. A similar experiment using E. coli ε results in full degradation of the DNA within 10 min. The greater activity of E. coli ε may be due to use of 37 °C for the exonuclease assays, which is probably suboptimal for the A. aeolicus ε subunit. The possibility that the A. aeolicus α contains an intrinsic 3′–5′-exonuclease activity was also tested in the exonuclease assay, but the result was negative (not shown). The presence of both polymerase and exonuclease activities suggests that recombinant A. aeolicus α and ε subunits are properly folded. Therefore, a lack of interaction between them is probably not due to one of them existing in a denatured state. Factors that may underlie the instability of A. aeolicus α and ε to form a complex are explored further under “Discussion.”

A. aeolicus α and τ Interact—In the E. coli system, the τ subunit forms a tight contact with the α subunit of the pol III core DNA polymerase, and this complex can be detected by gel filtration analysis at even low concentrations (100 nM) (46). Our studies in the S. pyogenes system demonstrate that the S.
**A** Western

**B** Quantitation

---

**Fig. 5.** *A. aeolicus* contains $\tau$ but not $\gamma$. *A. aeolicus* whole cells were analyzed by Western blot to determine whether a truncated ($\gamma$) product of dnaX is present or if only full-length product ($\tau$) is observed. The position of *A. aeolicus* $\tau$ is noted on the left by comparison to the mobility of recombinant $\tau$ in an adjacent lane of the same gel (not shown). The position of size standards is shown to the right of the gel. B, the plot quantitates the amount of intracellular $\tau$ by comparison of the intensity of *A. aeolicus* $\tau$ in cells to the intensity of known amounts of pure recombinant *A. aeolicus* $\tau$ analyzed in the same gel. The table takes into consideration the number of *A. aeolicus* cells applied to lanes of the Western blot in A to arrive at the average number of molecules of $\tau$ (as trimer) per *A. aeolicus* cell.

Pyogenes $\tau$ subunit interacts with *S. pyogenes* pol C; however, the contact is weaker than in the *E. coli* system.

In Fig. 7, we examine the *A. aeolicus* $\tau$ and $\alpha$ subunits for interaction between them. In Fig. 7A, *A. aeolicus* $\alpha$-$\tau$ interaction was analyzed in the Elisa type assay as described above for analysis of $\alpha$-$\epsilon$. In this case, $A. aeolicus$ $\alpha$ is adhered to the wells, followed by blocking with nonspecific protein, and then addition of *A. aeolicus* $\tau$. Unbound $\tau$ was washed away, and antibody directed against *E. coli* $\tau$ (which cross-reacts with *A. aeolicus* $\tau$) was used to detect whether *A. aeolicus* $\tau$ had bound to $\alpha$. The results demonstrate a high signal of $\tau$ bound to the well treated with $\alpha$. No $\tau$ is detected if the well is not pretreated with *A. aeolicus* $\alpha$. Hence, *A. aeolicus* $\alpha$ and $\tau$ directly interact. Similar results were obtained using *E. coli* $\alpha$ and $\tau$ (also shown in Fig. 7A).

Next we designed another assay to examine *A. aeolicus* $\tau$ and $\alpha$ for interaction, and this second method carries the advantage of demonstrating that the interaction is functional. The assay is based on the fact that *E. coli* $\alpha$ has very low activity on a singly primed M13mp18 ssDNA template coated with SSB, but addition of *E. coli* $\tau$ provides over 10-fold stimulation. This stimulation of *E. coli* $\alpha$ is specific to *E. coli* $\tau$, as use of *S. pyogenes* $\tau$ does not stimulate the reaction (Fig. 7B). Likewise, *S. pyogenes* $\alpha$ is stimulated by *S. pyogenes* $\tau$, but not by *E. coli* $\tau$ (Fig. 7B). In keeping with interaction between *A. aeolicus* $\alpha$ and $\tau$, *A. aeolicus* $\alpha$ is stimulated by *A. aeolicus* $\tau$. We were unable to examine whether *E. coli* $\tau$ or *S. pyogenes* $\tau$ stimulate *A. aeolicus* $\alpha$ due to the 65 °C reaction temperature required by the *A. aeolicus* system for activity.

In Fig. 7C, *A. aeolicus* $\alpha$ and $\tau$ were analyzed for $\alpha$-$\tau$ complex formation by gel filtration on an FPLC Superose 12 column. The top two panels show the elution profiles of *A. aeolicus* $\alpha$ and $\tau$ alone, and the bottom panel shows the analysis of a mixture of $\alpha$ and $\tau$. The result shows that the *A. aeolicus* $\alpha$-$\tau$ complex cannot be isolated by gel filtration and therefore suggests that they do not form as tight of a complex as *E. coli* $\alpha$-$\tau$, which is stable to gel filtration even at concentrations below 0.1 $\mu$M. The concentrations of $\alpha$ and $\tau$ used in the analysis of Fig. 7C were 37 and 46 $\mu$M, respectively. We have also analyzed *A. aeolicus* $\alpha$ and $\tau$ at yet higher concentrations (150 and 92 $\mu$M, respectively) but still observe no interaction between them (data not shown).

*A. aeolicus* $\alpha$ Functions with the $\beta$ Clamp—Does *A. aeolicus* pol III $\alpha$ subunit function with *A. aeolicus* $\beta\delta\delta'$ and $\beta$? This is tested in the experiments of Fig. 8 in which a singly primed M13mp18 ssDNA circle (7.2 kb) is utilized as substrate. In the *E. coli* system, this large ssDNA circular primed template must be coated with SSB in order for it to serve as an efficient substrate for pol III holoenzyme. Without SSB, *E. coli* pol III holoenzyme is encumbered by DNA secondary structure which SSB mostly eliminates by virtue of its tight interaction with the ssDNA template, providing about a 10-fold stimulation in synthesis relative to the absence of SSB. However, with SSB present, the *E. coli* holoenzyme extends the primer completely around the circular template within 11 s at 30 °C to form the RFII species. In the absence of $\beta$, the *E. coli* core polymerase does not extend the primer more than a few hundred nucleotides (12). However, when $\beta$ is first assembled onto the primed site, core becomes rapid and processive in synthesis (5).

To develop this assay in the *A. aeolicus* system, we cloned the *A. aeolicus* ssb gene into pET11 and expressed and purified the SSB (a sample of the preparation is shown in lane 7 of Fig. 1). With the *A. aeolicus* SSB, $\beta$ clamp and $\gamma\delta\delta'$ clamp loader in hand, we examined their effect on the *A. aeolicus* pol III $\alpha$ subunit in extension of a primed site on the M13mp18 ssDNA genome. As a basis for comparison we first examined the behavior of $\alpha$ on singly primed SSB-coated M13mp18 ssDNA in the absence of $\beta$ subunit (Fig. 8A). In this experiment, product is labeled by polymerase-catalyzed incorporation of [$\alpha$-$32$P]deoxyribonucleoside triphosphate. Reactions were incubated at a temperature of 65 °C, as the experiment of Fig. 6 demonstrates that the A.
pol III Holoenzyme of Aquifex aeolicus

A) ELISA

B) DNA SYNTHESIS

C) GEL FILTRATION

M13mp18 ssDNA no matter the time or concentration of polymerase used due to presence of insurmountable barriers to extension (48). In fact, SSB inhibits chain extension by pol III  and pol III Holoenzyme of Aquifex aeolicus. SSB stimulates synthesis by pol I, but not pol II, in the absence of SSB (48).

Next, the time course of DNA synthesis by pol III of pol III core in the absence of subunit was examined in the presence of both α and β clamp (Fig. 8B). The results demonstrate formation of complete RFII product within 2 min at all concentrations of α. At the highest amount of α used, formation of the RFII product occurs...
within 1 min indicating a speed of at least 120 nucleotides per s. Indeed, at the two highest \( \alpha \) concentrations, the synthetic time course is about the same indicating that the reaction is saturated with respect to \( \alpha \) at 2.64 \( \mu \)g and above. At \( \alpha \) subunit concentrations lower than 0.88 \( \mu \)g, less RFII product is formed at the 1-min time point compared with the 2-min time point. Furthermore, the immature products formed at 15 and 30 s become distinctly shorter as the \( \alpha \) subunit is titrated downward. The dependence of chain length on \( \alpha \) concentration indicates that \( \alpha \) does not remain attached to the \( \beta \) clamp over the synthesis of the entire 7.2-kb circle. In other words, processivity of the \( \alpha \) subunit with the \( \beta \) clamp is less than 7 kb. Most likely, \( \beta \) remains tightly bound to DNA continuously as \( \alpha \) jumps from one \( \beta \)-containing template to the next during synthesis. This behavior is similar to that of the E. coli system when \( \alpha \) subunit is used with \( \beta \) and \( \gamma \) complex instead of core or \( \alpha \gamma \) complex (9). In this case, the \( \beta \) clamp confers a processivity of \( \sim 1-3 \) kb onto \( \alpha \), and the speed of the pol III \( \alpha \beta \) complex is about 300 nucleotides per s at 37 °C (compared with about 1 kb/s when \( \alpha \gamma \) complex is used at the same temperature).

The A. aeolicus pol III Replicase Is Thermostable—Next, we examined the thermostability of the A. aeolicus pol III replication system and its rate of synthesis at different temperatures. These next experiments were performed using subsaturating pol III \( \alpha \) subunit in order to assess which temperature is most favorable, and therefore the rates of polymerization are less than the maximal rates observed in Fig. 8. The experiments were also performed in either the presence of SSB (a) or its absence (b). Proteins were preincubated with the DNA substrate for 2 min with two dNTPs to allow time for protein assembly onto DNA, and then synchronous synthesis was initiated upon addition of the remaining two dNTPs. Timed aliquots were quenched, and the products were analyzed in alkaline and native agarose gels. First we consider below the reactions that were performed in the presence of SSB.

At temperatures between 60 and 75 °C, the A. aeolicus system yields significant levels of synthesis, and as the temperature is elevated, the rate of synthesis increases (Fig. 9A). Very little synthesis is observed at 55 °C, which is presumably too low a temperature for the workings of this complex machinery from an extreme thermophile. The inactivity at low temperature may be ascribed, at least in part, to inactivity of the pol III \( \alpha \) polymerase, as simple gap filling assays using \( \alpha \) alone showed low activity at reduced temperature (see Fig. 6B). It remains possible that the clamp loading operation is also diminished at low temperature. The A. aeolicus SSB, however, would appear

![Fig. 8. Activity of the A. aeolicus pol III replicase.](image-url)
to remain functional at low temperature as it stimulates the E. coli pol III system at 37 °C (data not shown).

Synthetic activity is greatly reduced at 80 °C indicating that one or more components of the A. aeolicus pol III replicase denature at this temperature. DNA polymerase assays on gapped DNA at different temperatures also indicate that A. aeolicus pol III α subunit loses activity at 80 °C (Fig. 6B) suggesting that α is the inactive component. However, it remains possible that yet other A. aeolicus components also lose activity at 80 °C and higher. The thermostability of these proteins will be examined below. It is also possible that the primed template may lose efficiency in supporting synthesis at these high temperatures. In these experiments, we utilize a DNA 30-mer primer, the Tm of which is below 80 °C. However, we have raised the primer concentration in the assays to increase the occupancy of the primer on the ssDNA template at elevated temperature. To determine optimal primer concentration, we have titrated primer into reactions at different temperatures. The experiments shown here utilize 2 μM primer; higher concentrations gave no greater synthesis at any temperature. We have also titrated a 90-mer DNA oligonucleotide primer into the assay at different temperatures but observed slightly lower activity than that obtained using the DNA 30-mer.

In the E. coli system, the primary role of SSB on synthesis is thought to be removal of ssDNA secondary structure. Temperature can also remove secondary structure in ssDNA. It therefore seems reasonable to expect that SSB may not have as great of a stimulatory effect on synthesis at high temperature compared with a lower temperature. Ability of the A. aeolicus replication system to function above 60 °C, where some secondary structure should melt without SSB, provides the opportunity to assess the effect of SSB on synthesis at high temperature. The replication reactions in Fig. 9B are identical to those in Fig. 9A, except that instead of adding SSB, only the ssDNA buffer is added to the reactions. The comparison demonstrates that synthesis in the absence of SSB is about the same as in its presence, consistent with the idea that SSB stimulates polymerase by removing secondary structure.

The temperature stability of each replicase component was determined in the experiment of Fig. 10. Each individual subunit, or complex (as indicated in each panel), was incubated for 2 min at elevated temperature in the absence of other components and then was shifted to ice before being assayed for activity. The thermostability of each component was tested under several different buffer conditions containing either 0.1% Triton X-100, 0.01% Nonidet P-40, 0.05% Tween 20, 4 mM CaCl2, 40% glycerol, or combinations of these reagents. Heat-treated subunits were tested for activity by combining them with the other (unheated) subunits in the primed M13mp18 ssDNA synthesis assay. The assays contained α, ρδδ′, and β (one of which was temperature-treated) and were conducted at 70 °C for 1.5 min. Because this assay does not depend on the presence of SSB, we assayed the temperature stability of A. aeolicus SSB by substituting it for E. coli SSB in assays at 37 °C using E. coli α, ρδδ′, and β subunit.

The results in Fig. 10 show that A. aeolicus β and SSB are by far the most thermostable components (B and D). Seventy five percent or more of their activity was retained under all conditions tested, even at 90 °C. The ρδδ′ clamp loader was also highly thermostable provided it was heat-treated in buffers lacking Tween (C). The a DNA polymerase was the most heat-sensitive of the A. aeolicus pol III replicase components (A). Under most conditions, the α subunit lost 25–50% activity upon incubation at 80 °C and lost 80–100% activity upon 90 °C treatment. However, polymerase activity was largely stabilized in the presence of 4 mM CaCl2 and 40% glycerol, retaining over 50% activity after incubation at 90 °C. As expected from the thermostability results of α and ρδδ′, an equimolar mixture of α and ρδδ′ was generally thermostable, but was most stabilized to heat treatment by 4 mM CaCl2 and 40% glycerol (E).

**DISCUSSION**

**Similarities to the E. coli pol III Replicase**

The results of this study confirm that bacteria that grow at very high temperatures utilize the same overall strategy for processive replication as is observed in the mesophile, E. coli. Hence, A. aeolicus utilizes a pol III polymerase that is tethered to DNA by a β sliding clamp which, in turn, requires a clamp loader complex for assembly onto DNA. Previous studies of thermophilic DNA polymerases have focused mainly on homologues to DNA polymerase I (reviewed in Ref. 62). However, recent studies (35, 36) demonstrated the presence of γ and τ
proteins in a thermophile which suggested they may use a multicomponent replicase like E. coli pol III holoenzyme for chromosomal replication. Since those studies, the genomes of several thermophilic organisms have been sequenced. These genomes contain at least some of the genes encoding subunits homologous to subunits of the E. coli pol III holoenzyme. This report confirms the presence of a working pol III machinery in E. coli core. The current study also demonstrates that A. aeolicus αβ (plus αε) is not fully processive during primer extension around M13mp18, again like E. coli αβ. Studies in the E. coli system demonstrate that the ε proofreader is needed for optimal performance of the α subunit (9). Whereas E. coli pol III core and αε complex function with β to synthesize DNA at a speed of about 1 kb/s (at 37 °C), with a processivity greater than the 7.2-kb M13mp18 genome, the α subunit travels with β at a speed of about 300 nucleotides/s with a processivity of 1–3 kb (9). Hence, ε binding to α would appear to result in a more active form of the polymerase. These comparisons suggest that A. aeolicus α may also bind another protein(s) to form a pol III “core” that has similar speed and processivity with β as E. coli core.

Differences to the E. coli pol III Replicase

Subunit Interactions—It is widely anticipated that the polymerase and proofreading exonuclease activities will be tightly associated in chromosomal replicases because of the need for high fidelity to accurately duplicate an entire genome. Although A. aeolicus α and ε interact, the expectation that A. aeolicus α and ε would form a tight αε complex, as in the E. coli system, was not met. Because replicases across the evolutionary spectrum contain or have tightly associated proofreading exonuclease activity, it seems likely that A. aeolicus αε forms a tight complex in vivo. For example, the higher temperature at which A. aeolicus grows may strengthen the interaction between these two proteins. Alternatively, another protein may be required in A. aeolicus which serves as a brace, binding to both α and ε, thereby stabilizing the αε contact (neither α nor ε serves this function; data not shown). Finally, α and ε may associate tightly when present on a primed template.

Another difference to the E. coli system is the relatively low...
affinity interaction between *A. aeolicus* α and τ. Although we show herein that *A. aeolicus* α and τ directly interact, they do not form a complex that can be isolated by gel filtration as is readily demonstrated for *E. coli* ατ complex (32). In the *E. coli* system the C-terminal sequences of τ are required for binding to α and the DnaB helicase (32–34, 40). In this regard, the *A. aeolicus* τ subunit is only 54.3 kDa, significantly less than *E. coli* τ (71.1 kDa) and only 7 kDa larger than *E. coli* γ (47.5 kDa), and this discrepancy between the length of *E. coli* τ and *A. aeolicus* τ is predominantly in the C-terminal region. For example, the N-terminal 40 kDa of *A. aeolicus* τ contains the greatest extent of the homology to *E. coli* τ and corresponds to the sequences needed to bind δ, δ', and ATP for motor protein function. However, it remains possible that *A. aeolicus* ατ interaction increases at the high temperatures where this thermophile lives.

SSB—The SSB generally stimulates its cognate DNA polymerase. For example, T4 gp32 protein, *E. coli* SSB, and eukaryotic RPA all significantly enhance DNA synthesis by their respective DNA polymerase holoenzyme. The basis for this enhancement by SSB is generally believed to be the elimination of DNA secondary structure “road blocks” to polymerase translocation. Theoretically, SSB should slow polymerase chain extension, because some of the energy of nucleotide polymerization must be expended to displace tightly bound SSB from template ssDNA during its conversion to duplex. If SSB displacement is rate-limiting, or partially rate-limiting, its displacement will slow the intrinsic rate of DNA synthesis. If this is the case, the observed SSB stimulation of synthesis is probably due to elimination of kinetic barriers (i.e. DNA hairpins) that exert much more effect on polymerase speed than the barrier of SSB displacement. Thus, the net effect of substituting the SSB displacement barrier for the DNA secondary structure barrier is a stimulation of synthetic rate.

At high temperatures many DNA secondary structures may spontaneously melt. Therefore, for organisms that grow at high temperature, the DNA melting role of SSB may be less significant to polymerase progression. Indeed, the results of this report demonstrate that at elevated temperature, where most significant polymerase enhancement by SSB is generally believed to be the elimination of DNA polymerase progression. The evolution of both translational and transcriptional mechanisms to produce γ(*E. coli* and *T. thermophilus*, respectively) would suggest that the truncated product plays an important role in the organisms that produce it. However, genetic studies show that the dnaX gene in *E. coli* can be mutated such that the truncated product is not produced, with no growth defects (58). This *dnaX* mutant strain should make the truncated product from *dnaX*. The evolution of both translational and transcriptional mechanisms to produce γ(*E. coli* and *T. thermophilus*, respectively) would suggest that the truncated product plays an important role in the organisms that produce it. However, genetic studies show that the dnaX gene in *E. coli* can be mutated such that γ is not produced, with no growth defects (58). This *dnaX* mutant strain should make only the τ complex clamp loader, in which the three γ subunits are replaced by three τ subunits. This should also have the effect of dedicating the clamp loader to the replication fork because the C-terminal domains of τ will connect the clamp loader to core polymerases and the replicative DnaB helicase.

The fact that some bacteria have evolved frameshifting strategies to produce γ suggests that there is some role(α) for a γ complex clamp loader. For example, β clamps must not only be

A search of the *A. aeolicus* genome does not yield a significant match to *E. coli* θ, and in fact a search of the entire GenBank™ yields only one significant match to θ.²

In common with the genomic sequences of most other organisms, *A. aeolicus* shows no significant sequence matches to the *E. coli* χ and ψ subunits of the clamp loader within DNA pol III holoenzyme. Biochemical studies in the *E. coli* system show that these subunits are not required for clamp loading action (46, 54). What do these subunits do? The ψ subunit binds to γ and increases the stability of γε complex and the γδδ complex (54, 55). The χ subunit binds to ϕ in the γ complex clamp loader (54). The χ subunit also binds to SSB (56, 57). The χ-to-SSB contact is involved in displacement of primase from the RNA-primed site, thereby helping the polymerase and primase to trade places on the RNA primer (51). This χ-SSB interaction also aids clamp loading under conditions of elevated ionic strength, and stimulates polymerase elongation as well (56, 57). A PsiBlast search of GenBank™ using *E. coli* χ and ψ sequences as queries shows a somewhat broader distribution of homologues to these two proteins among different bacterial species, although the list is still confined to only a few organisms.³ It remains quite possible that yet other organisms may contain homologues to these two subunits, but sequence changes, combined with their small size, may preclude their identification by sequence comparison. This scenario is underscored by the difficulty of recognizing the essential δ subunit that is present apparently in all bacteria but difficult to identify by sequence searches.

**Lack of γ Subunit—**Examination of *dnaX* genes in GenBank™ shows that the *dnaX* gene of some bacteria contains a frameshift sequence but several others do not. *A. aeolicus dnaX* contains neither the ribosomal translational frameshift sequence nor the transcriptional slippage sequence. Consistent with this, we demonstrate in this report that only the full-length protein, designated τ, is detected by Western analysis of *A. aeolicus* whole cell extracts. Production of only the full-length product of *dnaX* is by no means unique as we have shown previously (38) that only τ is produced from *dnaX* in the Gram-positive organism, *S. pyogenes* (as recombinant protein in *E. coli*).

The genes encoding clamp loader subunits in the T4 phage, yeast, human, and archaeal systems are not known to produce a truncated product. Hence, it may be more appropriate to ask why *E. coli*, and some other bacteria, go through the trouble of producing a truncated product from *dnaX*. The evolution of both translational and transcriptional mechanisms to produce γ(*E. coli* and *T. thermophilus*, respectively) would suggest that the truncated product plays an important role in the organisms that produce it. However, genetic studies show that the *dnaX* gene in *E. coli* can be mutated such that γ is not produced, with no growth defects (58). This *dnaX* mutant strain should make only the τ complex clamp loader, in which the three γ subunits are replaced by three τ subunits. This should also have the effect of dedicating the clamp loader to the replication fork because the C-terminal domains of τ will connect the clamp loader to core polymerases and the replicative DnaB helicase.

**Similarities to Other Replicases**

Absence of χ, ψ, and θ Subunits—Besides replicate subunits that play catalytic roles in clamp loading and polymerization, the *E. coli* DNA pol III holoenzyme contains three small subunits that are not absolutely required for these processes. The smallest of these, θ (8.6 kDa), associates directly with ε subunit in the heterotrimeric pol III core polymerase (53). Absence of θ shows no defect in polymerase action or function of polymerase with the clamp loader and clamp in vitro (9, 53). Consistent with this, the *holE* gene encoding θ can be deleted without noticeable consequence to *E. coli* growth and viability (11). Moreover, homologues to θ are not widespread among bacteria.

---

² The PsiBlast search of GenBank™ identifies a homologue to *E. coli* θ in *Pasteurella multocida*.

³ The PsiBlast search of GenBank™ identifies homologues to both *E. coli* χ and ψ in *Hemophilus influenzae*, *Pasteurella multocida*, and *Vibrio cholerae*. In addition, χ homologues were present in *Pseudomonas aeruginosa*, *Xylella fastidiosa*, *Neisseria meningitides*, and *Caulobacter crescentus*.
Thermophilic pol III and Its Use in Technology—Current DNA amplification techniques make use of relatively distributive single subunit DNA polymerases whose physiological role is likely to be more similar to the repair enzyme, DNA polymerase I, than a chromosomal replicase. It seems quite likely that a rapid pol III-type enzyme endowed with a sliding clamp for much higher speed, processivity, and fidelity would provide advantages over polymerases of a more distributive type. For example, a rapid and processive thermophilic DNA replicase may yield larger products in “long chain PCR” applications and could perhaps deliver more reliable performance in these procedures. The PCR format cycles the reaction through a high temperature DNA denaturing step. Except for α, the components of the A. aeolicus pol III replicase are quite thermostable and would likely withstand these high temperature treatments. The α subunit is the most thermostable component and is less capable of withstanding temperatures used in polymerase chain cycling reactions. However, A. aeolicus α can withstand temperatures in the 50–80 °C range and thus may function well in high temperature isothermal amplification methods.

We have recently made the discovery in the E. coli system that β functions with numerous proteins, including DNA polymerase I (59). Hence, it may very well be possible to derive benefit upon addition of the highly thermostable A. aeolicus β clamp and γδ clamp loader to current PCR protocols utilizing thermophilic DNA polymerase I homologues. These components should be capable of withstanding the repeated cycles through 95 °C in the PCR procedure. Technological applications of these thermophilic replication components to new and existing procedures will be an exciting prospect for future study.

REFERENCES
1. Kuriyan, J., and Onrust, R. (1993) J. Mol. Biol. 234, 915–925
2. Davey, M. J., and O’Donnell, M. (2000) Curr. Opin. Chem. Biol. 4, 581–586
3. Bruck, I., and O’Donnell, M. (2001) Genome Biol. 2, 1–3
4. Kong, X. P., Onrust, R., O’Donnell, M., and Kuriyan, J. (1992) Cell 69, 425–437
5. Stukenberg, P. T., Studwell-Vaughan, P. S., and O’Donnell, M. (1991) J. Biol. Chem. 266, 11328–11334
6. Kelman, Z., and O’Donnell, M. (1993) Annu. Rev. Biochem. 64, 223–289
7. Maki, H., and Kornberg, A. (1985) J. Biol. Chem. 260, 12897–12902
8. Scheuermann, R. H., and Echols, H. (1985) Proc. Natl. Acad. Sci. U.S.A. 84, 7747–7751
9. Studwell, P. S., and O’Donnell, M. (1990) J. Biol. Chem. 265, 1171–1178
10. McHenry, C. S., and Crow, W. (1979) J. Biol. Chem. 254, 1748–1753
11. Slater, S. C., Lifisco, M. R., O’Donnell, M., and Maurer, R. (1994) J. Bacteriol. 176, 815–821
12. Fritsche, J., Johanson, K. O., McHenry, C. S., and Bambara, R. A. (1982) J. Biol. Chem. 257, 5692–5699
13. Onrust, R., and O’Donnell, M. (1993) J. Biol. Chem. 268, 17166–17172
14. Dong, Z., Onrust, R., Skangalis, M., and O’Donnell, M. (1993) J. Biol. Chem. 268, 17178–17185
15. O’Donnell, M., Onrust, R., Dean, F. B., Chen, M., and Hurwitz, J. (1993) Nucleic Acids Res. 21, 1–3
16. Neuwald, A. F., Aravind, L., Spouge, J. L., and Koonin, E. V. (1999) Genome Res. 9, 23–47
17. Gaasterland, T., Young, W. G., Lenox, A. L., and Ferrari, M. E. (1994) J. Biol. Chem. 269, 13365–13371
18. Stukenberg, P. T., Turner, J., and O’Donnell, M. (1999) EMBO J. 18, 771–783
19. Stewart, J., Hingorani, M. M., Kelman, Z., and O’Donnell, M. (2001) J. Biol. Chem. 276, 19182–19189
20. Brinton, K., Onrust, R., Fang, L., and O’Donnell, M. (1995) J. Biol. Chem. 270, 13358–13365
21. Stukenberg, P. T., Turner, J., and O’Donnell, M. (1999) Cell 96, 877–886
22. Stukenberg, P. T., Turner, J., and O’Donnell, M. (2000) EMBO J. 18, 5131–5144
23. Assen, B., Brinton, K., Hingorani, M. M., Beechem, J. M., O’Donnell, M. F., and Bloom, L. B. (2000) J. Biol. Chem. 275, 3006–3015
24. Flower, A. M., and McHenry, C. S. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 3713–3717
25. Brinton, K., Turner, J., and O’Donnell, M. (2001) J. Biol. Chem. 276, 4433–4440
26. Deckert, G., Warren, P. V., Gaasterland, T., Young, W. G., Lenox, A. L., Graham, D. E., Overbeek, R., Snead, M. A., Keller, M., Anjey, M., Huber, R., Feldman, R. A., Short, J. M., Olsen, G. J., and Swanson, R. V. (1998) Nature 392, 353–358
27. Turner, J., and O’Donnell, M. (1999) Methods Enzymol. 262, 442–449
28. Rowen, L., and Carniello, A. (1978) J. Biol. Chem. 253, 758–764
29. Kuriyan, J., and O’Donnell, M. (1995) Methods Enzymol. 262, 430–442
30. Stukenberg, P. T., Turner, J., and O’Donnell, M. (1994) Cell 78, 877–887
31. Onrust, R., Finkelstein, J., Turner, J., Naktinis, V., and O’Donnell, M. (1995) J. Biol. Chem. 270, 13366–13377
32. Pritchard, A. E., Dallmann, H. G., Glover, B. P., and McHenry, C. S. (2000) Nucleic Acids Res. 28, 6536–6545
33. LaDuca, R. J., Fay, P. J., Chuang, C. M., McHenry, C. S., and Bambara, R. A. (1983) Biochemistry 22, 5177–5188
34. Dallen, T. M., and Ferrari, M. F. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 1683–1688
35. Onrust, R., and O’Donnell, M. (2000) J. Biol. Chem. 275, 28971–28983
36. Stukenberg, P. T., Turner, J., and O’Donnell, M. (1994) Cell 78, 877–887
37. Onrust, R., Finkelstein, J., Turner, J., Naktinis, V., and O’Donnell, M. (1995) J. Biol. Chem. 270, 13366–13377
38. Turner, J., and O’Donnell, M. (1994) Methods Enzymol. 262, 442–449
39. Turner, J., and O’Donnell, M. (2004) Annu. Rev. Biochem. 63, 547–570
40. Studwell-Vaughan, P. S., and O’Donnell, M. (1995) J. Biol. Chem. 266, 11785–11791
41. Xiao, H., Dong, Z., and O’Donnell, M. (1993) J. Biol. Chem. 268, 11779–11784
42. Glover, B. P., and McHenry, C. S. (2000) J. Biol. Chem. 275, 3017–3020
43. Kuriyan, J., and O’Donnell, M. (1998) EMBO J. 17, 2436–2449
44. Stukenberg, P. T., Turner, J., and O’Donnell, M. (1994) Cell 78, 877–887
45. Lopez de Sario, F. J., and O’Donnell, M. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 11713–11718
46. Whelan, J., and O’Donnell, M. (1999) Cell 96, 153–163
47. Bloom, L. B., Goodman, M. F., and O’Donnell, M. E. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 6536–6545
48. petals, P. S., and O’Donnell, M. (1999) J. Biol. Chem. 275, 34609–34618
49. Vieille, C., and Zeikus, G. J. (2001) Microbiol. Mol. Biol. Rev. 65, 1–43
50. Vieille, C., and Zeikus, G. J. (2001) Microbiol. Mol. Biol. Rev. 65, 1–43
DNA: REPLICATION REPAIR AND RECOMBINATION:
Analysis of a Multicomponent
Thermostable DNA Polymerase III
Replicase from an Extreme Thermophile

Irina Bruck, Alexander Yuzhakov, Olga Yurieva, David Jeruzalmi, Maija Skangalis,
John Kuriyan and Mike O'Donnell
J. Biol. Chem. 2002, 277:17334-17348.

Access the most updated version of this article at http://www.jbc.org/content/277/19/17334

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 60 references, 40 of which can be accessed free at
http://www.jbc.org/content/277/19/17334.full.html#ref-list-1