Identification of the β-binding Domain of the α Subunit of Escherichia coli Polymerase III Holoenzyme*

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Rapid and processive DNA synthesis by Escherichia coli DNA polymerase III holoenzyme is achieved by the direct interaction between the α subunit of DNA polymerase III core and the β sliding clamp (LaDuca, R. J., Crute, J. J., McHenry, C. S., and Bambara, R. A. (1986) J. Biol. Chem. 261, 7550–7557; Stukenberg, T. P., Studwell-Vaughan, P. S., and O'Donnell, M. (1991) J. Biol. Chem. 266, 11328–11334). In this study, we localized the β-binding domain of α to a carboxyl-terminal region by quantifying the interaction of β with a series of α deletion proteins. Purification and binding analysis was facilitated by insertion of hexahistidine and short biotinylation sequences on the deletion terminus of α. Interaction of β with α deletion proteins was studied by gel filtration and surface plasmon resonance. α lacking 169 COOH-terminal residues still possessed β-binding activity; whereas deletion of 342 amino acids from the COOH terminus abolished β binding. Deletion of 542 amino acids from the NH2 terminus of the 1160 residue α subunit resulted in a protein that bound β 10–20-fold more strongly than native α. Hence, portions of α between residues 542 and 991 are involved in β binding. DNA binding to α apparently triggers an increased affinity for β (Naktinis, V., Turner, J. A., and O'Donnell, M. (1996) Cell 84, 137–145). Our findings extend this observation by implicating the amino-terminal polymerase domain in inducing a low affinity taut conformation in the carboxy-terminal β-binding domain. Deletion of the polymerase domain (or, presumably, its occupancy by DNA) relaxes the COOH-terminal domain, permitting it to assume a conformation with high affinity for β.

DNA polymerase III holoenzyme is the replicative polymerase responsible for the synthesis of the majority of the Escherichia coli chromosome. The special replicative role of DNA polymerase III is conferred by its ability to interact with other replication proteins. It apparently functions as an asymmetric dimer, capable of coordinating leading and lagging strand synthesis at the replication fork. Holoenzyme1 is highly processive (Fay et al., 1982; Wu et al., 1992), perhaps capable of synthesizing the entire E. coli chromosome without dissociation. The β subunit is required for processive DNA synthesis (Fay et al., 1982; LaDuca et al., 1986). A β dimer forms a donut-shaped structure around duplex DNA (Kong et al., 1992) and contacts the polymerase by direct protein-protein interactions (LaDuca et al., 1986; Stukenberg et al., 1991). These properties are consistent with a sliding clamp that can translocate with the polymerase and tether it to the DNA template. Mutations in dnaN, the structural gene for β, have been found that suppress mutations in dnaE, the gene encoding α, providing genetic support for an interaction between α and β (Kuwabara and Uchida, 1981).

β is assembled onto primed DNA templates by DnaX complex, either τ(τβδγψ) or γ(τδβγψ) complex (Wickner, 1976; Dallmann and McHenry, 1995; Onrust et al., 1995a). Both τ and γ are the products of the dnaX gene; τ is the full-length product of DnaX, and γ is the NH2-terminal two-thirds of DnaX, synthesized by a +1 translational frameshift (McHenry et al., 1989; Tsuchihashi and Kornberg, 1990; Blinkowa and Walker, 1990; Flower and McHenry, 1990). Both τ and γ contain an ATPase activity that is essential for β clamp loading onto primed templates (Lee and Walker, 1987; Tsuchihashi and Kornberg, 1989). Once the β clamp is loaded onto a DNA template forming a preinitiation complex, pol III associates to form a stable initiation complex that replicates DNA very rapidly and processively in the presence of four dNTPs (Wickner, 1976; O'Donnell, 1987).

The catalytic subunit of DNA polymerase III, α, in addition to its functional role, must serve as an organization protein to hold the 18-protein holoenzyme complex together (Dallmann and McHenry, 1995). τ binds the COOH-terminal region of α with high affinity (Kim and McHenry, 1996) to form a dimeric polymerase, pol III* (McHenry, 1982; Studwell-Vaughan and O'Donnell, 1991). The NH2-terminal half of α contains the polymerase active site.2 Because the high processivity of holoenzyme results from the direct interaction of α and β, a specific region of α must be devoted to binding β. Here, we identify the α domain responsible for interaction with β.

EXPERIMENTAL PROCEDURES

Proteins—α deletion proteins αNC11, αNC1542, αC10, αC148, αC169, and αC342 were purified as described (Kim and McHenry, 1996). The β subunit was prepared as described by Johanson et al. (1986), and the τ subunit was purified as described by Dallmann et al. (1995). Streptavidin was purchased from Pierce.

Materials and Buffers—CM5 sensor chip (research grade), N-hydroxysuccimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, ethanalamine-HCl (pH 8.0) and P-20 surfactant were purchased from Pharmacia Biosensor. Centricron-30 microconcentrators were obtained from Amicon. Poly(dA) and oligo(dT)12 were purchased from Pharmacia Biotech Inc. DE81 ion exchange papers (2.5 cm diameter) were from Whatman. Buffer TSG is 25 mM Tris-HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl, and 10% glycerol. Buffer T5 is 25 mM Tris-HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl, and 0.005% P-20 surfactant.

Superose 12 Gel Filtration Chromatography—A 24-ml Superose 12 FPLC gel filtration column (Pharmacia Biotech) was used to study the interaction of α and β as described (Stukenberg et al., 1991). βs (510

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1 The abbreviations used are holoenzyme, E. coli DNA polymerase III holoenzyme; pol III, DNA polymerase III core (α, ε, and δ); RU, resonance unit(s).

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V overnight. The gel was stained with Coomassie Brilliant Blue over-
Terminus Interacts Strongly with
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b
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(abmonomer(300 pmol αN542 and 510 pmol βi), αN542-β (300 pmol αN542 and 510 pmol βj), αC10 (150 pmol),
αC10 (200 pmol αC10 and 510 pmol βj), αC342 (150 pmol), αC342-β (300 pmol αC342 and 510 pmol βj), αC169 (200 pmol), αC169-β (275 pmol αC169 and 510 pmol βj), αC342 (200 pmol), and αC342-β (270 pmol αC342 and 510 pmol βj) in 200 μl of buffer TSG were incubated at room temperature for 30 min and injected individually onto the column at 4°C. 70 fractions (200 μl each) were collected at a flow rate of 0.1 ml/min and analyzed by SDS-gel electrophoresis. αC10, αC348, αC342, αC169, and αC342 were concentrated in a Centricon-30 microconcentrator at 5,000 × g at 0°C for 30 min before use.

SDS-Polyacrylamide Gel Electrophoresis—Superose 12 column fractions (70 μl) were loaded onto a 12.5% SDS-polyacrylamide gel (1.5 × 18 × 16 cm) prepared according to Laemmli (1970) and separated at 70 V overnight. The gel was stained with Coomassie Brilliant Blue over-
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Estimation of the Kd for the α-β Interaction by Gel Filtration—To
estimate the affinities of the interaction between β and the α deletion proteins using gel filtration analysis, stained gels of the column fractions were subjected to a laser densitometric scan (Molecular Dynamics). This interaction was modeled as a simple 1:1 interaction between α monomer and β dimer (α + α + β), where the dissociation constant for this reaction is defined as Kd = [α][β][αβ]. The molar concentration of bound α monomer ([αβ]) determined from the equation:

\[ K_d = \frac{([\alpha][\beta][\alpha\beta])}{([\alpha][\beta]_{total})} \]

where \( [\alpha] \) is the total molar concentration of α loaded onto the column, \( [\beta] \) is the total molar concentration of β, \( [\alpha\beta] \) is the total molar concentration of αβ, \( [\alpha\beta]_{total} \) is the total molar concentration of αβ, and \( [\alpha\beta]_{bound} \) is the total molar concentration of αβ bound to α.

Since significant dilution occurs during gel filtration, so that the eluted protein concentration is not equal to the protein concentration loaded onto the column, the Kd was also calculated with a correction factor derived from the ratio of the total elution volume of α (generally, 1 ml) to the injection volume (0.2 ml) that yields a 5-fold dilution. Thus, Kd was calculated assuming a 5-fold lower α and β concentrations than the actual initial molar input concentrations. The corrected and uncorrected Kd values represent upper and lower limits, respectively, providing a comparison of the relative affinity between β and the α deletion proteins since all filtration analyses were done under the same conditions.

BIAcore Binding Analysis—A BIAcore™ instrument (Pharmacia Bi-

β-stimulated DNA synthesis catalyzed by α deletion proteins on homopolymeric templates—To further examine the interaction between β and α deletion proteins (αC30, αC348, αC169, and αC342), β-stimulation of DNA synthesis by α deletion proteins on poly(dA)-oligo(dT) primer-templates was measured by the method of LaDuca et al. (1992). Homopolymeric templates were prepared by annealing poly(dA) and oligo(dT)primers (as nucleotide), 25 fmol of α or α deletion protein, 50 μM THDTP (400 cpm/μM), 10 mM MgCl2 in the presence of varying amounts of the β subunit (0–80 × 106 units) in enzyme dilution buffer (50 mM HEPES (pH 7.5), 200 μg/ml bovine serum albumin, 0.02% Nonidet P-40, 20% glycerol, and 10 mM dithiothreitol) were incubated at 37°C for 5 min and terminated by the addition of EDTA to a final concentration of 100 mM. Samples were filtered onto DE81, washed, and bound radioactivity determined as described (LaDuca et al., 1986).

RESULTS

α Containing a Deletion of 542 Amino Acids from the NH2 Terminus Interacts Strongly with β—We previously reported the construction of a series of plasmids that contain progressive deletions from the termini of α (Kim and McHenry, 1996). The terminus of the deletion-containing α subunit was fused to a peptide containing a hexahistidine sequence and a short sequence that is biotinylated in vivo, facilitating purification and immobilization (Kim and McHenry, 1996). We analyzed the ability of these deleted α subunits to interact with β2 using a gel filtration method initially developed by Stukenberg et al. (1991) to demonstrate an α-β interaction. As a positive control, the interaction of wild-type α and β was tested. β2 alone eluted mainly at fractions 61–63 (Fig. 1A), and α eluted at fractions 57–60 (Fig. 1B). When β was incubated with α prior to gel filtration, its elution position shifted to a higher molecular weight (fractions 56–58, Fig. 1C), indicating an interaction. The densitometric scan of this gel permitted estimation of an α-β Kd of 0.2 and 1 μM, consistent with the value 250 mM determined by Stukenberg et al. (1991), αN11 (missing the first amino acid and fused to a peptide) showed nearly the same interaction as wild-type α (Fig. 1, D and E, Table 1), suggesting that the fusion peptide itself did not perturb β binding. The α deletion protein αN542, which has a 542-amino acid deletion from the NH2 terminus, bound β about 20-fold more strongly than full-length α (Fig. 1, compare C with G) (Table 1). Formation of partially unfolded proteins that aggregated precluded examination of deletions greater than 542 residues (Kim and McHenry, 1996). These results showed that the β-binding site was located in the COOH-terminal half of the α subunit.
The COOH-terminal 169 Amino Acids of α Are Not Required for Interaction with β—To determine the COOH-terminal limit of α for β binding, we tested the β-binding activities of COOH-terminal deletion proteins. The full-length COOH-terminal α fusion protein (αCΔ0) showed very similar β-binding activity to that of wild-type α (Table I). β was shifted to the elution position of αCΔ0 due to their interaction (compare Fig. 1A with 2A and B). Both αCΔ48 and αCΔ169 bound β (Fig. 2, C–F), although binding was 12- and 30-fold weaker than that of wild-type α, respectively (Table I). Deletion of 342 amino acids from the COOH terminus resulted in a protein (αN342) that did not bind β (Fig. 2, G and H). Thus, residues 542–991 define the approximate terminal limits of an α domain involved in interaction with β.

Deletion of the Polymerase Domain of α Increases the Affinity of the COOH-terminal Domain for β—Gel filtration analysis suggested that αN542 bound β 20-fold stronger than full-length α. To verify this observation, we examined the interaction of αN542 with β by surface plasmon resonance. Biotinylated α deletion proteins αN1 and αN542 were immobilized on a sensor chip via streptavidin interaction as described (Kim and McHenry, 1996). When α derivatives were coupled at levels less than 1000 RU, the signal due to α-β binding was so small that it could not be reliably analyzed. Therefore, α deletion proteins were immobilized at a high level (3500 RU for αN542 and 3300 RU for αN1). Under these conditions, β bound and dissociated rapidly from αN1 and αN542 (Fig. 3, A and B). Even at high β concentrations (1 μM), binding was substoichiometric (0.08 β/αN1 and 0.2 β/αN542). The on-rate (k_on) and off-rate (k_off) of the interaction between αN1 and β were 5.1 × 10^3 s⁻¹ M⁻¹ and 7.5 × 10⁻² s⁻¹, respectively, resulting in a K_D of ~20701 nM (K_D = k_off/k_on). αN542 bound β with a K_D of ~40 nM (k_on = 9.5 × 10⁵ s⁻¹ M⁻¹; k_off = 3.6 × 10⁻² s⁻¹). Both K_D values were in the range determined by gel filtration analysis. The interaction of αN1-β was weaker than that of αN542-β, consistent with results from gel filtration analysis, even though there was only a 3.7-fold difference in the K_D by BIAcore analysis compared to a 20-fold difference in the K_D by gel filtration.

τ and β Can Both Bind to the COOH Terminus of α Simultaneously—Both τ and β bind to the COOH-terminal region of α (Kim and McHenry, 1996), raising the possibility of steric exclusion by virtue of overlapping binding sites of τ and β. To test this possibility, we performed gel filtration experiments in which αN1 and τ (4-fold molar excess) were incubated followed by addition of β. As shown in Fig. 4 (A and B), β was detected in early column fractions indicating an α-τ-β complex and thus the absence of significant competition between τ and β. Moreover, the binding of τ to α is much tighter than that of β (70 μM versus 0.2-1 μM, respectively), and would have prevented β binding if binding were competitive. τ binding to αN1 appeared to induce a slight decrease in the affinity between β and αN1, as indicated by a comparison to the gel filtration results with β-αN1 alone (Fig. 1E); indeed, densitometric scans of Fig. 1E and 4B showed that approximately 4-fold less β bound to αN1 in the presence of τ. When αN542 was used for gel filtration with τ and β, β was also observed to interact with this COOH-terminal domain of α in the presence of excess τ (Fig. 4C). We were unable to compare the affinity of the αN542-β interaction in the presence and absence of τ due to the similar molecular mass of τ and αN542 and their co-migration on polyacrylamide gels, although the level of β bound in the presence of τ (Fig. 4C) appears to be less than that observed in the absence of τ (Fig. 1G).

We also used surface plasmon resonance to determine whether τ affects the α-β interaction as indicated by gel filtration analysis. Streptavidin was immobilized as described (Kim and McHenry, 1996), followed by sequential injections of αN542, β (1), τ (1), β (2), τ (2), and β (3) over the streptavidin chip to examine the effect of τ on αN542-β binding (sensorgram 1 of Fig. 5). The interaction of αN542 and streptavidin resulted in immobilization of about 5000 RU. The injection of τ (1) over αN542 showed binding saturation since a second injection, τ (2), did not lead to additional τ binding (sensorgram 1 of Fig. 5). In the absence of τ, αN542 bound β (about 600 RU) with a K_D of ~80 nM (k_on = 6.3 × 10⁵ s⁻¹ M⁻¹; k_off = 4.8 × 10⁻² s⁻¹). αN542-τ complex also bound β (about 350 RU), with a slightly diminished K_D of ~200 nM (k_on = 5.6 × 10⁴ s⁻¹ M⁻¹; k_off = 0.1 s⁻¹), as determined from two β injections (β (2) and β (3)). Data were analyzed after subtraction of sensorgram 2 from sensorgram 1 (Fig. 5), which represents β binding to the
Deletion proteins capable of synthesizing DNA using a stoichiometric excess of the polymerase active site converts which DNA occupancy of the polymerase active site converts ... modulation of this interaction is important in permitting rapid polymerase-proficient α deletion proteins is adequate to permit β stimulation of polymerization.

DISCUSSION

The rapid and processive DNA synthesis by holoenzyme depends on the β sliding clamp (Fay et al., 1982). The DnaX complexes assemble β on DNA in a reaction that is coupled to ATP hydrolysis (Wickner, 1976; Onrust et al., 1991). The β sliding clamp confers high processivity by tethering polymerase to the DNA template (LaDuca et al., 1986; Stukenberg et al., 1991). In this report, we have identified the β-binding site of α by measuring the binding of β to modified α subunits that contain a deletion from either their NH₂ or COOH terminus. The results indicate that a region between amino acid residues 542 and 991 of α is involved in β binding.

Interestingly, deletion of 542 amino acids from the NH₂ terminus of α resulted in a 4–20-fold higher affinity for β compared to wild-type α. This suggests that the amino-terminal domain of α, which contains the polymerase active site, is an inhibitory domain for the binding of β. Recently, O'Donnell and colleagues (Naktinis et al., 1996) proposed a model in which DNA occupancy of the polymerase active site converts α to a form that has an increased affinity for β. Presumably, the modulation of this interaction is important in permitting rapid cycling of the lagging strand polymerase after completion of

α₅₄₂–τ complex without the background of τ dissociating from α₅₄₂. Hence, the interaction of α₅₄₂–β was 2-fold stronger before τ injection than after τ injection, as indicated by the Kᵦ values calculated from a single sensorgram and the mass amount of β bound to α₅₄₂.

Stimulation of DNA Synthesis of α Deletion Proteins by β—We detected interactions between β and α deletion proteins α₅₀₀, α₅₄₈ and α₅₁₆₉, but no interaction between β and α₅₄₂ using gel filtration analysis. DNA synthetic activity of pol III was reportedly stimulated 7-fold by the addition of a stoichiometric excess of β on poly(dA)-oligo(dT) primer-templates (LaDuca et al., 1986). Here we examined β stimulation of α deletion proteins capable of synthesizing DNA using homopolymeric templates as described under “Experimental Procedures.” The synthetic activities of three α deletion proteins α₅₀₀, α₅₄₈ and α₅₁₆₉ and wild-type α were stimulated about 3-fold in the presence of 10,000–40,000 units (0.4–1.6 × 10⁵ molar excess) of β (Fig. 6). The β subunit did not stimulate α₅₄₂ activity even in the presence of the same gap-filling units of α₅₄₂ as α₅₁₆₉. Thus, the maintenance of β binding activity in polymerase-proficient α deletion proteins is adequate to permit β stimulation of polymerization.
DNA synthesis stimulation of α deletion proteins by β. The DNA synthesis of α (■), αCΔ0 (●), αCΔ48 (▲), αCΔ169 (▼), and αCΔ342 (♦) was measured in the presence of various concentrations of β (0-80 \texttimes 10^{-4} \text{units}) on ppol(dA)-oligo(dT) templates (3:1 molar ratio of dA and dT) as described under "Experimental Procedures."

synthesis of an Okazaki fragment on the lagging strand. Our results are consistent with this proposal and add to our understanding of the mechanism. The unoccupied NH₂-terminal polymerase domain must constrain the carboxyl-terminal β binding domain to a taut low affinity conformation, since its removal permits the high affinity COOH-terminal domain to relax to a high β affinity conformer (Fig. 7A). Extending this observation to the model in which DNA modulates the binding of β by α, DNA occupancy of the polymerase site would alter its conformation so that the constraint on the COOH-terminal α domain is removed, permitting it to revert to a relaxed conformation with higher affinity for β (Fig. 7B). Upon completion of Okazaki fragment synthesis, and provided the affinity of the polymerase at a nick results in a lower DNA-polymerase affinity (a feature of the model that has not been verified quantitatively), dissociation of the polymerase from DNA would result in a lower affinity for β, permitting polymerase cycling. This model may be oversimplified since β presumably functions by tethering the polymerase to DNA, so that if the polymerase transiently dissociates during elongation, it would rapidly rebind. Such a mechanism would not be tenable if DNA dissociation automatically caused a break in the α-β link. Answers to these questions may rest in the relative rates of polymerase reassociation with a primer terminus versus β dissociation. A mechanism in which the conformational state of the polymerase influences complex stability may have other implications, including destabilization of elongating complexes to permit protein exchange during translesion synthesis in SOS mutagenesis.

Both τ and β bind to the carboxyl-terminal domain of α (Kim and McHenry, 1996; present study). However, the τ binding domain appears to be larger and more complex. Like β binding, τ binding is abolished by deletions beyond residue 542 from the αNαL542+complex without the background of τ simultaneously dissociating from αNαL542. The thick lines indicate binding of β to αNαL542-τ complex.

π binding

τ binding

366
542
812
991
1,160 a.a.

Pol III

active site

Polymerase

β binding

N

DnaE

C

A

low affinity

high affinity

R

O

O

B

low affinity

high affinity

R

O

C

r

FIG. 7. The NH₂-terminal polymerase domain of α, if not occupied by DNA, inhibits binding of β to the carboxyl-terminal domain. A, deletion of the polymerase domain (residues 1-542) of α results in a protein with enhanced affinity for β₂. The ring represents β₂; the shaded unlabeled parallelograms, and ovals represent the amino-terminal polymerase domain of α; T and R represent the carboxyl-terminal α domain in the taut and relaxed conformations, respectively. Double line represents DNA. B, DNA occupancy of the polymerase active site may permit it to change conformation, removing its constraints on the β-binding carboxyl-terminal domain of α, and permitting it to relax from the low affinity taut (T) conformer to the high affinity relaxed (R) conformer. Shaded parallelograms and ovals represent the amino-terminal polymerase domain of α. C, map of the domains of α.

FIG. 5. Effect of τ on the interaction between αNαL542 and β in BIAcore binding analysis. Streptavidin was coupled to the chip as described (Kim and McHenry, 1996), and αNαL542 was immobilized by streptavidin, resulting in immobilization of αNαL542 for sensorgram 1 and 4700 RU for sensorgram 2. All binding analyses were carried out in buffer T5 at a flow rate of 2 μL/min at 20 °C. Sensorgram 1 represents the sequential injection of αNαL542 (270 nM, 10 μL), β (1) (500 nM, 30 μL), τ (1) (500 nM, 10 μL), β (2) (500 nM, 10 μL), τ (2) (500 nM, 20 μL), and β (3) (500 nM, 10 μL). Sensorgram 2 represents the sequential injection of αNαL542 (270 nM, 10 μL), β (1) (500 nM, 30 μL), and τ (2) (500 nM, 20 μL). The sensorgram at the bottom of the figure shows the subtraction of sensorgram 2 from sensorgram 1 to represent β binding to αNαL542-τ complex without the background of τ simultaneously dissociating from αNαL542. The thick lines indicate binding of β to αNαL542-τ complex.
amino terminus. However, $\tau$ binding is also abolished by small deletions (48 residues) from the carboxyl terminus, whereas deletions of up to 169 amino acids from the COOH terminus are tolerated without loss of $\beta$ binding (Fig. 7C). The mapping in Fig. 7C is approximate and only defines the terminal sequences involved in forming a stable domain that permits protein-protein interaction. The actual number of amino acids within these domains involved in the interactions is probably much smaller.

Present models for holoenzyme function assume simultaneous $\tau$ and $\beta$ binding during elongation. In fact, the $\gamma$ subunit that is linked to $\tau$ in holoenzyme (Dallmann and McHenry, 1986; Onrust et al., 1995a) cross-links to primers in an initiation complex at a site between $\beta$ and $\alpha$ (Reems et al., 1995). We find that $\beta$ binds to both free $\alpha$ and $\alpha-\tau$ with approximately the same affinity, although the affinity for $\beta$ may be slightly diminished by the presence of $\tau$.

The close proximity of $\tau$ and $\beta$ may permit modulation of the $\alpha-\beta$ interaction by other mechanisms that are sensitive to the state of the primosome, a component that is linked to the holoenzyme via a $\tau$-DnaB interaction (Kim et al., 1996a). DnaB serves a dual role as the cellular helicase that separates two parental strands at the replication fork (LeBowitz and McMacken, 1986) and as a mobile promoter that interacts with parental strands at the replication fork (LeBowitz and McMacken, 1986). These contacts may enable a mechanism whereby synthesis of a new primer is communicated to the newly elongating polymerase, perhaps further destabilizing it for cycling to the new primer. Interestingly, $\tau$ also functions to protect $\beta$ on elongating polymerases, preventing its premature removal by $\gamma$ complex (Kim et al., 1996b). The conformational state of $\tau$ could influence this property. Proof of these hypotheses awaits precise identification of the interaction domains of the replisome subunits and analysis of the strength and functional consequences of altered conformational states.

REFERENCES

Blinkow, A. L., and Walker, J. R. (1990) Nucleic Acids Res. 18, 1725–1730
Dallmann, H. G., and McHenry, C. S. (1995) J. Biol. Chem. 270, 29563–29569
Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1995) J. Biol. Chem. 270, 29555–29562
Fay, P. J., Johnson, K. O., McHenry, C. S., and Bambara, R. A. (1982) J. Biol. Chem. 257, 5692–5699
Flower, A. M., and McHenry, C. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3713–3717
Johnson, K. O., Haynes, T. E., and McHenry, C. S. (1986) J. Biol. Chem. 261, 11460–11465
Kim, D. R., and McHenry, C. S. (1996a) J. Biol. Chem. 271, 20690–20698
Kim, S., Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1996a) Cell 84, 643–650
Kim, S., Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1996b) J. Biol. Chem. 271, 4315–4318
Kong, X., Onrust, R., O'Donnell, M., and Kuriyan, J. (1992) Cell 69, 425–437
Kuwabara, N., and Uchida, H. (1983) Proc. Natl. Acad. Sci. U. S. A. 78, 5764–5767
LaDuca, R. J., Crute, J. J., McHenry, C. S., and Bambara, R. A. (1986) J. Biol. Chem. 261, 7550–7557
Laemmli, U. K. (1970) Nature 227, 680–685
LeBowitz, J. H., and McMacken, R. (1986) J. Biol. Chem. 261, 4738–4748
Lee, S. H., and Walker, J. R. (1987) Pro. Natl. Acad. Sci. U. S. A. 84, 2713–2717
McHenry, C. S. (1982) J. Biol. Chem. 257, 2657–2663
McHenry, C. S., Gries, M., Tomaszewicz, H., and Bradley, M. (1989) in Mechanisms in DNA Replication and Recombination (Richardson, C., and Lehman, I. R., eds) pp. 115–126, Alan R. Liss, Inc., New York
Mcmacken et al., 1984; Lebowitz and McMacken, 1986; Onrust et al., 1995a)
Nakitis, V., Turner, J., and O'Donnell, M. (1996) Cell 84, 137–145
O'Donnell, M. (1987) J. Biol. Chem. 262, 16558–16565
Onrust, R., Stukenberg, P. T., and O'Donnell, M. (1991) J. Biol. Chem. 266, 21681–21686
Onrust, R., Finkelstein, J., Nakitis, V., Turner, J., Fang, L., and O'Donnell, M. (1995a) J. Biol. Chem. 270, 13348–13357
Onrust, R., Finkelstein, J., Turner, J., Nakitis, V., and O'Donnell, M. (1995b) J. Biol. Chem. 270, 13366–13377
Reems, J. A., Wood, S., and McHenry, C. (1995) J. Biol. Chem. 270, 5606–5613
Studwell-Vaughan, P. S., and O'Donnell, M. (1991) J. Biol. Chem. 266, 19833–19841
Stukenberg, P. T., Studwell-Vaughan, P. S., and O'Donnell, M. (1991) J. Biol. Chem. 266, 11328–11334
Tongu, K., Peng, H., Marians, K. J. (1994) J. Biol. Chem. 269, 4675–4682
Tsuzuki, Z., and Kornberg, A. (1989) J. Biol. Chem. 264, 17790–17795
Tsuzuki, Z., and Kornberg, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2516–2520
Wickner, S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3511–3515
Wu, C. A., Zechner, E. L., and Marians, K. J. (1992) J. Biol. Chem. 267, 4030–4044