The Human Lagging Strand DNA Polymerase δ Holoenzyme Is Distributive*

Received for publication, July 24, 2012, and in revised form, August 20, 2012. Published, JBC Papers in Press, August 31, 2012, DOI 10.1074/jbc.M112.404319

Zhenxin Hu, Senthil K. Perumal, Hongjun Yue, and Stephen J. Benkovic†
From the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Background: Human DNA Pol δ holoenzyme carries out the lagging strand DNA synthesis.
Results: Human DNA Pol δ holoenzyme is distributive with nucleotide incorporation rates a hundredfold slower than its prokaryotes/phage counterparts.
Conclusion: Human DNA Pol δ holoenzyme dissociates and reassembles during DNA replication.
Significance: Probing the characteristics of Pol δ holoenzyme is important for elucidating its mechanistic details and function in vivo.

Polymerase δ is widely accepted as the lagging strand replicative DNA polymerase in eukaryotic cells. It forms a replication complex in the presence of replication factor C and proliferating cell nuclear antigen to perform efficient DNA synthesis in vivo. In this study, the human lagging strand holoenzyme was reconstituted in vitro. The rate of DNA synthesis of this holoenzyme, measured with a singly primed ssM13 DNA substrate, is 4.0 ± 0.4 nucleotides. Results from adenosine 5'- (3-thiotriphosphate) tetralithium salt (ATPγS) inhibition experiments revealed the nonprocessive characteristic of the human DNA polymerase (Pol δ) holoenzyme (150 bp for one binding event), consistent with data from chase experiments with catalytically inactive mutant Pol δAA. The ATPase activity of replication factor C was characterized and found to be stimulated ~10-fold in the presence of both proliferating cell nuclear antigen and DNA, but the activity was not shut down by Pol δ in accord with rapid association/dissociation of the holoenzyme to/from DNA. It is noted that high concentrations of ATP inhibit the holoenzyme DNA synthesis activity, most likely due to its inhibition of the clamp loading process.

Coordination of the activities of replicative polymerase and accessory proteins is indispensable for the replication of chromosomal DNA in different organisms. This process requires different proteins and enzymes to form a multimeric functional replisome to secure accurate and fast transfer of genetic information from one generation to the next (1, 2). DNA replication is semiconservative, and the leading strand synthesis is continuous, whereas the lagging strand is replicated in small fragments (Okazaki fragments) in a discontinuous fashion with intervening short stretches of DNA then sealed by DNA ligase (3). Unlike bacteriophages or prokaryotes, eukaryotic organisms encode two distinctive replicative polymerases to perform leading and lagging strand DNA synthesis (4). Recent investigations revealed that polymerase ε (Pol ε) and polymerase δ (Pol δ) carry out leading and lagging strand replication, respectively, in Saccharomyces cerevisiae (4). Several lines of evidence suggest that other eukaryotic organisms also encode two replicative DNA polymerases to duplicate their genomic DNA (4–6). The discrete Okazaki fragment size in eukaryotic cells is 100–250 bp long, implying that a slow and distributive lagging strand holoenzyme is sufficient to achieve the synthesis of short stretches of DNA. However, recently, Langston and O’Donnell (7) revealed that the S. cerevisiae Pol δ holoenzyme is fast and processive and invoked a collision model for the release and recycling of the lagging replicative polymerase. Previous in vitro reconstitution of Pol δ holoenzyme with proteins purified from calf thymus provided details about the components and characteristics of the replicative complex in vertebrates (8, 9). With singly primed M13, the synthesized products spanned a broad spectrum of primer extension fragments formed with apparent velocities of 1.6–5.0 nt/s (8, 9), much slower than those encountered with phage and bacterial polymerases.

The human Pol δ holoenzyme is generated from Pol δ, PCNA (sliding clamp), and RFC (clamp loader). Human Pol δ contains a catalytic subunit p125 and three auxiliary subunits p66, p50, and p12 (10). RFC is a heteropentameric complex, composed of one large subunit and four small subunits, and an ATP-dependent AAA⁺ ATPase that loads PCNA onto DNA (11). PCNA, a homotrimeric clamp, encircles the DNA substrate and is loaded at the primer/template (P/T) junction by RFC, providing a platform for the formation of the holoenzyme. The heterotrimeric RPA (single-stranded DNA (ssDNA)-binding protein) binds to ssDNA to remove secondary structures and prevent endonucleolytic cleavage of the DNA (12). All these components are necessary for optimal DNA synthesis (13). Recently, human lagging strand holoenzyme was reconstituted in vitro, and its processivity was compared with Pol ε (14). Here we report detailed measurements of the kinetics and processivity of this holoenzyme.

* This work was supported, in whole or in part, by National Institutes of Health Grant RO1 GM13307 (to S.J.B.).
† This article contains supplemental Figs. S1–S5 and Table S1.
To whom correspondence should be addressed: 414 Wartik Laboratory, Dept. of Chemistry, The Pennsylvania State University, University Park, PA 16802. Tel.: 814-865-2882; Fax: 814-865-2973; E-mail: sjb1@psu.edu.

The abbreviations used are: Pol, DNA polymerase; RFC, replication factor C; PCNA, proliferating cell nuclear antigen; RPA, replication factor A; ATPγS, adenosine 5’-(3-thiotriphosphate) tetralithium salt; P/T, primer/template; ssM13, single-stranded M13; nt, nucleotides; nt/s, nucleotides per second.
In this work, the human Pol δ holoenzyme complex was assembled in vitro, and the DNA replication reaction velocity and processivity were examined. The ATPase activities of RFC were measured to deduce the assembly pathway of the holoenzyme. Surprisingly, the dNMP incorporation rate of the reconstituted holoenzyme showed a negative correlation with the ATP concentration used in holoenzyme assembly.

**EXPERIMENTAL PROCEDURES**

The primers were synthesized by Integrated DNA Technologies. [α-32P]dATP, [γ-32P]ATP, and [α-32P]dCTP were purchased from PerkinElmer Life Sciences. DNA markers and T4 polynucleotide kinase were purchased from New England Biolabs. Polyethyleneimine-modified cellulose (PEI cellulose) TLC plates were obtained from EMD Biosciences. ATPγS was purchased from Calbiochem. dNTPs were from Denville Scientific Inc. Electrophoresis grade agarose was from IBI Scientific. Chromatography paper DE81 was from Whatman International Ltd.

**Plasmids and DNA Substrates**—The plasmids encoding the wild-type human Pol δ were generously provided by Yuji Masuda (13). The plasmid encoding the C-terminal His6-tagged wild-type subunits of human RPA was a kind gift from Marc Wold (12). The plasmids encoding the subunits of wild-type RFC were a generous gift from Paul Modrich (16). ssM13mp18 DNA was prepared by standard procedures. A catalytically inactive mutant, Pol δΔΔ (D755A/D757A mutant), was constructed by site-directed mutagenesis (forward primer, 5′-GGT GCG TGG GCA TTC GTC GCG CAG GCT CAG CGT-3′), and confirmed by sequence analysis.

The DNA substrate for measuring the RFC ATPase activity was prepared by annealing the template (5′-GGG TTT TCC CAG TCA CGA GGT TGT AAA ACG-3′) with a generous gift from Paul Modrich (16). ssM13mp18 DNA was prepared by standard procedures. A catalytically inactive mutant, Pol δΔΔ (D755A/D757A mutant), was constructed by site-directed mutagenesis (forward primer, 5′-GGT GCG TGG GCA TTC GTC GCG CAG GCT CAG CGT-3′), and confirmed by sequence analysis.

**Proteins**—The wild-type Pol δ was expressed and purified as described earlier (13). The Pol δΔΔ mutant was expressed and purified similarly to the wild-type enzyme. RPA and PCNA were expressed as reported previously (12, 15). Purifications of the wild-type RFC and truncated RFC are described elsewhere.3

**ATP Hydrolysis Assay**—Measurement of the intrinsic ATPase activity of RFC was carried out with various concentrations of ATP (0–1 mM) supplemented with [α-32P]ATP in the standard complex reaction buffer (25 mM Tris acetate (pH 7.8), 125 mM KOAc, 10 mM Mg(OAc)2, and 1 mM DTT) at 37 °C. The ATPase activity of the RFC complex in the presence of PCNA and/or DNA was examined in the presence of 1 mM ATP. When DNA was included in the reaction, the forked DNA described above with an end blocked with a biotin-avidin complex was utilized as a substrate to prevent PCNA from sliding away once loaded. Aliquots of the reactions were drawn at various time points and quenched with 0.5 mM EDTA (pH 8.0), and 1 μl of the quenched solution was spotted on a PEI cellulose plate and developed in 200 mM potassium P1 (pH 7.5) buffer, dried, and exposed to a PhosphorImager screen. The data were analyzed and quantified by ImageQuant software.

**ssM13 DNA Primer Extension Assay**—Each of the components of ssM13 DNA replication by human Pol δ holoenzyme was varied to determine the maximal dNMP incorporation rate. Replication assays were carried out in the standard complex reaction buffer at 37 °C. The 38-nucleotide-long primer (5′-GGG TTT TCC CAG TCA CGA GGT TGT TGT AAA ACG ACG GCC AG-3′, which is complementary to the 6328–6291 region of ssM13mp18 DNA) was annealed to ssM13 DNA at a ratio of 2:1. In the instances where 32P-labeled primer was used, the ratio of primer to template was 1:1:1. The primer was radio-labeled with 32P at the 5′-end by T4 polynucleotide kinase using standard procedures (17). A typical reaction contained 10 nM singly primed ssM13 DNA, 100 nM Pol δ, 100 nM RFC, 200 mM PCNA, 2 μM RPA, 50 μM dNTPs (10 μCi of [α-32P]dCTP was added), and 1 mM ATP in the standard complex reaction buffer that contained 10 mM Mg2+ to provide a standard reaction solution. The reactions were quenched with 2× loading buffer (60 mM NaOH, 2 mM EDTA) at regular intervals, and the products were resolved on a 0.8% alkaline agarose gel, transferred to DE81 paper, exposed to a PhosphorImager screen, and quantified by ImageQuant. The incorporation of radionucleotide into the product of the primer extension ssM13 DNA was calculated from a calibration curve. The ionic strength was normalized to 200 mM salt by the addition of NaCl.

For the ATPγS addition experiment, a premixed standard reaction mixture was incubated with primed ssM13 DNA for 1 min, and various concentrations of ATPγS were added to the reaction solution with [α-32P]dCTP. The reactions were chased at different times (Δt), quenched by the quenching buffer, resolved, and quantified as above.

For the polymerase Pol δΔΔ chase experiment, a premixed standard reaction solution was incubated with primed ssM13 DNA for 1 min, and then different concentrations of Pol δΔΔ were added to the reaction solution with [α-32P]dCTP. The reactions were chased at different times (Δt), quenched by the quenching buffer, resolved, and quantified as above.

**Polymerase Dilution Experiment**—The primer extension reaction contained 10 nM singly primed ssM13 DNA (the primer is labeled with 32P at the 5′-end), 50 nM Pol δ, 100 nM RFC, 200 mM PCNA, 2 μM RPA, 50 μM dNTPs, and 1 mM ATP in standard complex reaction buffer. After an incubation at 37 °C for 1 min, the reaction was diluted with the primer extension reaction mixture that lacked both the Pol δ and the primed ssM13 DNA to obtain 2-, 4-, and 8-fold dilutions of the reactions. The reactions were continued, aliquots were drawn at regular intervals, and the products were resolved on an alkaline agarose gel and quantified as described above.

**Monte Carlo Simulation of Dilution Experiment**—The Monte Carlo simulation for singly primed ssM13 DNA synthesis was done as described elsewhere.4 Briefly, the program tests the possibility of the presence of a holoenzyme on a DNA sub-

---

3 S. K. Perumal and S. J. Benkovic, manuscript in preparation.

4 H. Yue, M. M. Spiering, D. Chen, T.-H. Lee, and S. J. Benkovic, manuscript in preparation.
strate by comparing a computer-generated random number with an association/dissociation probability calculated based on the binding kinetics of the holoenzyme. If there is a holoenzyme, in a simulation step, the DNA is elongated by one nucleotide. The simulation step size, $\Delta t$, was 0.01 s, equal to the reciprocal of the single nucleotide incorporation rate of 100 nt/s (18). The best results were achieved when the association rate constant was set to $1 \times 10^6$ M$^{-1}$ s$^{-1}$ and the dissociation rate constant was set to 0.7 s$^{-1}$. A 7-min reaction was performed for each DNA substrate. During the first minute, the concentration of Pol$\delta$ was 50 nM, and over the next 6 min, the rate constant decreased by the same factor as the dilution in the corresponding experiments. A total of 0.5 million DNA molecules were simulated.

**RESULTS**

**ATPase Activity of RFC and Its Complexes**—Human RFC is a member of the AAA$^+\,$ superfamily of ATPases and utilizes ATP to initiate the assembly of the holoenzyme complex by loading PCNA onto the DNA substrate (11). To understand how the complex forms, we examined the ATPase activity of RFC under various conditions, and the results are shown in Table 1. The intrinsic ATPase activity of RFC is low, and the data fit to a Michaelis-Menten equation to yield a $k_{cat}$ of $0.065 \pm 0.01$ s$^{-1}$ and a $K_{m(\text{ATP})}$ of $57 \pm 6$ mM. All the other ATPase assays were carried out at a constant ATP concentration of 1 mM. End-blocked forked DNA stimulated the ATPase activity of RFC 4-fold to a $k_{cat}$ of $0.27 \pm 0.03$ s$^{-1}$ and an apparent $K_{m(\text{DNA})}$ of $27 \pm 7$ nM. PCNA had no effect on ATPase activity of RFC in the absence of DNA. In the presence of 250 nM RFC, 250 nM PCNA, 250 nM DNA, and 1 mM ATP, the ATP hydrolysis increased to an apparent $k_{cat}$ of $0.68 \pm 0.06$ s$^{-1}$. The addition of Pol$\delta$ had no significant effect on the ATPase activity of RFC.

**DNA Replication Dependence on ATP Concentration**—The ATPase activity of RFC showed a constant hydrolysis of ATP under different conditions, suggesting that PCNA was loaded onto the DNA P/T junction continuously even in the presence of human Pol$\delta$. To further understand whether the loading of PCNA is required after the initiation of DNA replication by Pol$\delta$ holoenzyme, we carried out an ATP concentration-dependent DNA replication experiment after the optimization of replication assay (supplemental Fig. S1). As shown in Fig. 1, when 0.5 or 1 mM ATP was used, the frontier velocity (length of the longest product divided by reaction time) was 15 nt/s (lanes 2 and 5); however, it dropped slightly to 13 nt/s (lane 8) when 2 mM ATP was used. In the presence of 4 mM ATP (lane 8), the velocity was reduced to 6 nt/s, and it was reduced to 2.5 nt/s when 6 mM ATP was added to the reaction (lane 11).

**DNA Replication Dependence on ATPγS and Rescue by ATP**—Unlike the bacteriophage T4 system where the ATPase activity of the clamp loader is dramatically reduced when the replicative polymerase was added to form a holoenzyme complex composed of stoichiometric clamp and polymerase (19, 20), Pol$\delta$ had no effect on the ATP hydrolysis activity of RFC. This phenomenon suggests that RFC might load PCNA onto the P/T

---

**TABLE 1**

| Component(s)          | $k_{cat}$ s$^{-1}$ |
|-----------------------|-------------------|
| RFC                   | 0.065 ± 0.010     |
| RFC + PCNA            | 0.070 ± 0.010     |
| RFC + DNA             | 0.27 ± 0.030      |
| RFC + PCNA + DNA      | 0.68 ± 0.060      |
| RFC + PCNA + DNA + Pol$\delta$ | 0.75 ± 0.070      |
junction of DNA continuously in an ATP-dependent manner. To test this hypothesis, the standard reaction was initiated and carried out for 1 min, chased with different concentrations of ATP$\gamma$S with $[^{32}\text{P}]$dCTP, and then quenched at different time points. The rates of nucleotide incorporation into the extension of primer of ssM13 DNA were measured and shown in Fig. 2. A control experiment verified that ATP$\gamma$S cannot support the formation of a catalytically competent holoenzyme (supplemental Fig. S2, lanes 2–6). When no ATP$\gamma$S was present, the reaction velocity was 4.0 nt/s, which agreed well with a recent study (14). In the presence of 1, 3, and 5 mM ATP$\gamma$S, the nucleotide incorporation rates were 2.0 ± 0.2, 1.2 ± 0.1, and 0.3 ± 0.1 nt/s, respectively. With increasing concentrations of ATP$\gamma$S, DNA synthesis gradually decreased with formation of shorter products. The DNA synthesis was proportional to the ratio of [ATP]/([ATP] + [ATP$\gamma$S]) as shown in Fig. 3. A control experiment in the absence of RFC showed that the DNA synthesis was negligible (supplemental Fig. S3, lane 2). The rescue experiment results showing that the ATP$\gamma$S-mediated inhibition of DNA synthesis was relieved by increasing the concentrations of ATP are shown in supplemental Fig. S2, lanes 7–16.

**DNA Replication Chased with Pol δAA**—To examine the processivity with human Pol δ holoenzyme and its dissociation from DNA, a catalytically inactive Pol δAA mutant was prepared to chase the standard reaction as was carried out previously in the T4 phage system (21) and is shown in Fig. 3. The rate of dNMP incorporation decreased with increasing amounts of the chase mutant (Fig. 3). When no Pol δAA was added, the DNA synthesis rate was 3.9 ± 0.3 nt/s; however, when an equal amount of Pol δAA was added to chase the reaction, the velocity dropped to 2.0 ± 0.2 nt/s. When a 2-fold excess of the mutant was used, the rate was further reduced to 0.4 ± 0.1 nt/s. No
Human Lagging Strand DNA Pol δ Holoenzyme Is Distributed

DNA synthesis was detected when a 4-fold excess of Pol δΔAA was used to chase the reaction. Theoretically, the DNA synthesis rates were calculated as 4.0, 2.0, 1.3, and 0.5 nt/s when no or 1-, 2-, or 4-fold dead mutant chased the standard reaction, as shown in gray in Fig. 3. The chase experiment with 4-fold excess of Pol δΔAA was carefully examined for DNA products (supplemental Fig. S5) and revealed a processivity of 150 ± 50 bp.

Human Polymerase δ Holoenzyme Is Not Processive—The polymerase chase experiment showed that the human Pol δ holoenzyme was not stable and that reassembly of the holoenzyme was indispensable for continued incorporation of nucleotide to extend the primer. The low resolution of the chase experiments precluded the accurate determination of the processivity of human Pol δ holoenzyme. To measure the processivity, we conducted the polymerase dilution experiment as well as its simulation by Monte Carlo methods (Fig. 4 and supplemental Fig. S4). The DNA products became shorter when the reaction was diluted 2-, 4-, and 8-fold. The simulation results indicated that the weak association and fast dissociation rate constants are responsible for the distributive behavior of human Pol δ holoenzyme. The calculated processivity is 140 bp (Table S1), which is in excellent agreement with the experimentally determined value of 150 ± 50 bp as noted above. The calculated velocity of the holoenzyme is 6.6 nt/s, which is consistent with the measured rate above (4.0 ± 0.4 nt/s).

DISCUSSION

It is estimated that the length of Okazaki fragments is 100–250 bp in eukaryotic cells, suggesting that a rapid and highly processive lagging strand replicative complex is not necessary. However, the S. cerevisiae Pol δ holoenzyme has a processivity greater than 5 kb (7) and an approximated rate of 60–150 nt/s (7, 22, 23). To the contrary, the human Pol δ holoenzyme appears to act distributively on an ssM13 template (13, 24–27). In this study, we measured in vitro these parameters and other properties of DNA synthesis by human Pol δ holoenzyme in detail.

PCNA Is Loaded onto P/T Junction Continuously—To understand how the human Pol δ holoenzyme forms, ATP hydrolytic activities of RFC with different complexes were examined to elucidate the pathway of Pol δ holoenzyme assembly. RFC, by itself, showed very low background ATP hydrolysis activity (0.065 ± 0.01s⁻¹). Upon the addition of a DNA substrate, the ATPase activity was stimulated 4-fold and further stimulated 2.5-fold (0.68 ± 0.06s⁻¹) by adding an equal amount of PCNA (250 nm). In the T4 bacteriophage holoenzyme formation, when the DNA polymerase (gp43) was introduced, the maximal ATPase activity was shut down to a background level, accounted for by a stable clamp-polymerase holoenzyme formation (19, 20). However, when human Pol δ was added to the PCNA loading system, the ATPase activity remained unchanged, indicating that the Pol δ-PCNA complex is labile and rapidly exchanges.

ATP Is Necessary to Maintain the Holoenzyme Complex—ATPγS has been reported to load PCNA in a nonproductive fashion and to prevent the RFC reloading process (11, 13, 28). To confirm that RFC is required for combined PCNA loading after the preassembly of Pol δ holoenzyme, ATPγS was added to the standard reaction. The DNA synthesis experiments were carried out under optimized conditions of protein concentrations (supplemental Fig. S1). With increasing concentrations of ATPγS, the DNA synthesis rate decreases and fits the theoretical curve \((V = V_o \times [ATP]/([ATP] + [ATPγS]))\) very well, suggesting that ATPγS binds to RFC with similar affinity as ATP and that the binding is reversible. The inhibition by ATPγS was rescued, when provided with enough ATP (supplemental Fig. S2). Our result is not consistent with an earlier report that 40-fold ATPγS had no effect on DNA synthesis after the preassembly of calf thymus Pol δ holoenzyme (29), but agrees well with the results from an experiment with the human Pol δ holoenzyme by Masuda et al. (13) that demonstrated the complete arrest of DNA primer elongation in the presence of ATPγS. The concentration-dependent inhibition by ATPγS demonstrated that ATP hydrolysis is necessary for the full activity of human Pol δ holoenzyme in DNA synthesis that acts primarily through fueling the activity of RFC to continually load PCNA.

Pol δ Readily Dissociates from Holoenzyme—To detect exchange between Pol δ polymerase as DNA-bound holoenzyme and Pol δ polymerase in solution, Pol δΔAA was utilized as a trap to chase the primer extension reaction. If the polymerase of bound Pol δ holoenzyme freely exchanges with solution polymerase, the rate should fit the equation, \(V = V_o \times [Pol \delta]/([Pol \delta] + [Pol \deltaΔAA])\) as shown in Fig. 3. However, if the holoenzyme resists exchange with Pol δΔAA, the velocity should be higher than that predicted by the free exchange equation. The chase experiment results showed that Pol δΔAA was a very efficient trap, and the rates are even lower than the prediction for the active exchange model, suggesting that Pol δΔAA might bind DNA more tightly than that of the wild type. The Pol δΔAA chase results, combined with RFC ATPase assays, demonstrate that the Pol δ holoenzyme is not stable and apt to dissociate and reassemble. The polymerase dilution experiment and the simulation data (Fig. 4 and supplemental Fig. S4) further support the dissociative behavior of human Pol δ holoenzyme. The association and dissociation constants of Pol δ at a DNA P/T (Table S1) junction suggest a fast equilibrium between formation and decomposition of the replicative complex, rendering a slow and distributive holoenzyme. Recently, Smith and Whitehouse (30) observed a coupling between lagging strand synthesis and chromatin assembly in S. cerevisiae, which functions as a brake to terminate a processive lagging strand holoenzyme. However, the in vitro experiment on human lagging strand synthesis shows it is distributive, suggesting that the length of Okazaki fragments is an intrinsic property of human Pol δ polymerase and does not depend on Flap endonuclease FEN1 and Dna2 helicase/nuclease, enzymes that are necessary for Okazaki fragment maturation (31–34).

High Concentrations of ATP Inhibit Holoenzyme Assembly and DNA Synthesis Activity—Another interesting result is the inhibition of DNA synthesis by high concentrations of ATP. In the formation of the holoenzyme, ATP is utilized by RFC to load PCNA onto a P/T junction. Generally, with saturating amounts of ATP, high concentrations would have no effect on PCNA loading and subsequent DNA synthesis. However, the frontier velocity of DNA synthesis dropped when the ATP con-
centration was increased from 0.5 to 6 mM. This is the first study demonstrating that high concentrations of ATP decreased the DNA incorporation rate by human Pol δ holoenzyme. The negative correlation between ATP concentration and holoenzyme activity is most likely due to the inhibition of the RFC loading process, similar to inhibition by ATP of clamp loader activity in the T4 bacteriophage system (35).

In conclusion, the human lagging strand polymerase δ in complex with PCNA manifests low intrinsic catalytic activity and processivity in vitro. This property is commensurate with the known size distribution of Okazaki fragments. The accompanying high dissociation rate of the PCNA clamp protein consequently imposes a continuous loading activity on the RFC clamp loader and a high demand for ATP. These measurements serve as a basis for evaluating the effect of additional protein components of the human replisome on DNA replication.

Acknowledgments—We thank Drs. Danqi Chen and Mark Hedglin for thoughtful discussion and critical reading of the manuscript.
REFERENCES

1. Benkovic, S. J., Valentine, A. M., and Salinas, F. (2001) Replisome-mediated DNA replication. Annu. Rev. Biochem. 70, 181–208

2. Pomerantz, R. T., and O’Donnell, M. (2007) Replisome mechanics: insights into a twin DNA polymerase machine. Trends Microbiol. 15, 156–164

3. Rossi, M. L., Purohit, V., Brandt, P. D., and Bambara, R. A. (2006) Lagging strand replication proteins in genome stability and DNA repair. Chem. Rev. 106, 453–473

4. Pursell, Z. F., Isoz, I., Lundström, E. B., Johansson, E., and Kunkel, T. A. (2007) Yeast DNA polymerase ε participates in leading strand DNA replication. Science 317, 127–130

5. Chilkova, O., Stenlund, P., Isoz, I., Stith, C. M., Grabowski, P., Lundström, E. B., Burgers, P. M., and Johansson, E. (2007) The eukaryotic leading and lagging strand DNA polymerases are loaded onto primer ends via separate mechanisms but have comparable processivity in the presence of PCNA. Nucleic Acids Res. 35, 6588–6597

6. Johansson, E., and Macneill, S. A. (2010) The eukaryotic replicative DNA polymerases take shape. Trends Biochem. Sci. 35, 339–347

7. Langston, L. D., and O’Donnell, M. (2008) DNA polymerase δ is highly processive with proliferating cell nuclear antigen and undergoes collision release upon completing DNA. J. Biol. Chem. 283, 29525–29531

8. Podust, V. N., Georgaki, A., Strack, B., and Hübscher, U. (1992) Calf thymus RF-C as an essential component for DNA polymerase δ and ε holoenzymes function. Nucleic Acids Res. 20, 4159–4165

9. Podust, V. N., and Hübscher, U. (1993) Lagging strand DNA synthesis by calf thymus DNA polymerases α, β, δ, and ε in the presence of auxiliary proteins. Nucleic Acids Res. 21, 841–846

10. Liu, L., Mo, J., Rodriguez-Belmonte, E. M., and Lee, M. Y. (2000) Identification of a fourth subunit of mammalian DNA polymerase δ. J. Biol. Chem. 275, 18739–18744

11. Yao, N. Y., Johnson, A., Bowman, G. D., Kuriyan, J., and O’Donnell, M. (2006) Mechanism of proliferating cell nuclear antigen clamp opening by replication factor C. J. Biol. Chem. 281, 17528–17539

12. Henrickson, L. A., Umbricht, C. B., and Wold, M. S. (1994) Recombinant replication protein A: expression, complex formation, and functional characterization. J. Biol. Chem. 269, 11121–11132

13. Masuda, Y., Suzuki, M., Piao, J., Gu, Y., Tsumoto, T., and Kamiya, K. (2007) Dynamics of human replication factors in the elongation phase of DNA replication. Nucleic Acids Res. 35, 6904–6916

14. Bermudez, V. F., Farina, A., Raghavan, V., Tappin, L., and Hurwitz, J. (2011) Studies on human DNA polymerase ε and GINS complex and their role in DNA replication. J. Biol. Chem. 286, 28963–28977

15. Maga, G., and Hübischer, U. (1995) DNA polymerase ε interacts with proliferating cell nuclear antigen in primer recognition and elongation. Biochemistry 34, 891–901

16. Kadyrov, F. A., Genschel, J., Fang, Y., Penland, E., Edelmann, W., and Modrich, P. (2009) A possible mechanism for exonuclease 1-independent eukaryotic mismatch repair. Proc. Natl. Acad. Sci. U.S.A. 106, 8495–8500

17. Yang, J., Zhuang, Z., Roccasceca, R. M., Traskelis, M. A., and Benkovic, S. J. (2004) The dynamic processivity of the T4 DNA polymerase during replication. Proc. Natl. Acad. Sci. U.S.A. 101, 8289–8294

18. Meng, X., Zhou, Y., Lee, E. Y., Lee, M. Y., and Frick, D. N. (2010) The p12 subunit of human polymerase δ modulates the rate and fidelity of DNA synthesis. Biochemistry 49, 3545–3554

19. Berdis, A. J., and Benkovic, S. J. (1997) Mechanism of bacteriophage T4 DNA holoenzyme assembly: the 44/62 protein acts as a molecular motor. Biochemistry 36, 2733–2743

20. Sexton, D. J., Kaboord, B. F., Berdis, A. J., Carver, T. E., and Benkovic, S. J. (1998) Dissecting the order of bacteriophage T4 DNA polymerase holoenzyme assembly. Biochemistry 37, 7749–7756

21. Zhuang, Z., Johnson, R. E., Haracska, L., Prakash, L., Prakash, S., and Benkovic, S. J. (2008) Regulation of polymerase exchange between Pol η and Pol δ by monoubiquitination of PCNA and the movement of DNA polymerase holoenzyme. Proc. Natl. Acad. Sci. U.S.A. 105, 5361–5366

22. Brewer, B. J., Zakian, V. A., and Fangman, W. L. (1980) Replication and meiotic transmission of yeast ribosomal RNA genes. Proc. Natl. Acad. Sci. U.S.A. 77, 6739–6743

23. Fangman, W. L., and Brewer, B. J. (1991) Activation of replication origins within yeast chromosomes. Annu. Rev. Cell Biol. 7, 375–402

24. Conti, C., Saccà, B., Herrick, J., Lalou, C., Pommier, Y., and Bensimon, A. (2007) Replication fork velocities at adjacent replication origins are coordinately modified during DNA replication in human cells. Mol. Biol. Cell 18, 3059–3067

25. Poli, J., Tsaponina, O., Crabbé, L., Keszthelyi, A., Pantesco, V., Chabes, A., Lengronne, A., and Pasero, P. (2012) dNTP pools determine fork progression and origin usage under replication stress. EMBO J. 31, 883–894

26. Courbet, S., Gay, S., Arnoult, N., Wronka, G., Anglana, M., Brison, O., and Debatisse, M. (2008) Replication fork movement sets chromatin loop size and origin choice in mammalian cells. Nature 455, 557–560

27. Elvers, I., Johansson, F., Groth, P., Erixon, K., and Hellday, T. (2011) UV stalled replication forks restart by re-priming in human fibroblasts. Nucleic Acids Res. 39, 7049–7057

28. Gomes, X. V., Schmidt, S. L., and Burgers, P. M. (2001) ATP utilization by yeast replication factor C. II. Multiple stepwise ATP binding events are required to load proliferating cell nuclear antigen onto primed DNA. J. Biol. Chem. 276, 34776–34783

29. Podust, V. N., Podust, L. M., Müller, F., and Hübischer, U. (1995) DNA polymerase δ-holoenzyme: action on single-stranded DNA and on double-stranded DNA in the presence of replicative DNA helicases. Biochemistry 34, 5003–5010

30. Smith, D. I., and Whitehouse, I. (2012) Intrinsic coupling of lagging strand synthesis to chromatin assembly. Nature 483, 434–438

31. Burgers, P. M. (2009) Polymerase dynamics at the eukaryotic DNA replication fork. J. Biol. Chem. 284, 4041–4045

32. Garg, P., Stith, C. M., Sabour, N., Johansson, E., and Burgers, P. M. (2004) Idling by DNA polymerase δ maintains a ligatable nick during lagging strand DNA replication. Genes Dev. 18, 2764–2773

33. Kao, H. I., and Bambara, R. A. (2003) The protein components and mechanism of eukaryotic Okazaki fragment maturation. Crit. Rev. Biochem. Mol. Biol. 38, 433–452

34. Turchi, J. J., Huang, L., Murante, R. S., Kim, Y., and Bambara, R. A. (1994) Enzymatic completion of mammalian lagging strand DNA replication. Proc. Natl. Acad. Sci. U.S.A. 91, 9803–9807

35. Pietroni, P., and von Hippel, P. H. (2008) Multiple ATP binding is required to stabilize the “activated” (clamp open) clamp loader of the T4 DNA replication complex. J. Biol. Chem. 283, 28338–28353