Role of the Core DNA Polymerase III Subunits at the Replication Fork

α IS THE ONLY SUBUNIT REQUIRED FOR PROCESSIVE REPPLICATION*

(Received for publication, August 4, 1997, and in revised form, November 3, 1997)

Kenneth J. Marians‡, Hiroshi Hiasa‡, Deok Ryong Kim§, and Charles S. McHenry§

From the ‡Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 and the §Department of Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262

The DNA polymerase III holoenzyme is composed of 10 subunits. The core of the polymerase contains the catalytic polymerase subunit, α, the proofreading 3′ → 5′ exonuclease, ε, and a subunit of unknown function, θ. The availability of the holoenzyme subunits in purified form has allowed us to investigate their roles at the replication fork. We show here that of the three subunits in the core polymerase, only α is required to form processive replication forks that move at high rates and that exhibit coupled leading- and lagging-strand synthesis in vitro. Taken together with previous data this suggests that the primary determinant of replication fork processivity is the interaction between another holoenzyme subunit, τ, and the replication fork helicase, DnaB.

The replisome of *Escherichia coli* is a complex protein machine composed of the DNA polymerase III holoenzyme (pol III HE),1 which synthesizes the nascent DNA, and the primosome, which unwinds the parental duplex and synthesizes primers for the initiation of Okazaki fragment synthesis (1). The composition of the primosome can vary depending on the manner in which the replication fork helicase, DnaB (2), is introduced to the DNA. Primosomes loaded at oriC are composed of only DnaB and DnaG (3), the primase (4), whereas primosomes loaded at recombination intermediates (5) are likely to also include PriA, PriB, PriC, and DnaT (6, 7). The function of these latter four proteins at the replication fork have yet to be established.

The pol III HE is itself composed of 10 subunits (8). α, the catalytic polymerase subunit (9), ε, the 3′ → 5′ proofreading exonuclease (10), and θ, a subunit of unknown function, associate to form the polymerase core (11). α binds ε but not θ, whereas θ binds ε but not α (12), suggesting a linear array of αεθ. The association of α and ε acts to improve the catalytic efficiency of each polypeptide, increasing the polymerase activity of α by 2–3-fold (13, 14), and the exonuclease activity of ε by 8-fold on a mispaired substrate and 32-fold on a paired substrate (15). It was also suggested that ε increased the processivity of α during DNA synthesis on primed single-stranded DNA (15).

The DnaX complex is composed of six subunits organized as τ₂γδδψ (16, 17). τ acts to dimerize two core assemblies via an interaction with α (18, 19). At the replication fork, this results in a physical coupling of the leading- and lagging-strand polymerases in space (20). The other subunits in the DnaX complex, which have been referred to as the γ complex (21), are likely involved in loading and unloading the processivity subunit, β (22, 23), from the DNA (24, 25). The β dimer encircles the DNA and associates with α to topologically lock the polymerase onto the template, thereby enabling processive synthesis (26, 27). τ also plays a central role at the replication fork where, as a result of a protein-protein interaction with DnaB, it cements the replisome together, allowing rapid replication fork movement (28). This interaction also results in the protection of β on the leading-strand side from premature recycling by the γ-complex (29), thus defining which of the two polymerase cores becomes the leading-strand polymerase at the replication fork (29, 30).

We have been studying the action of the pol III HE at replication forks formed during rolling circle DNA replication on specialized tailed form II (TFII) DNA templates in the presence of the single-stranded DNA-binding protein (SSB) and the δX-type primosomal proteins. In this report, we have analyzed the contributions of the three subunits of the polymerase core to replication fork function. We find that only the α subunit is required to form replication forks that are processive and which move at the same rate as those formed with the intact HE. In addition, replication forks containing only α responded to control of Okazaki fragment synthesis as mediated by the primase (31–33), elaborated coupled leading- and lagging-strand synthesis, and were as stable as replication forks formed with the complete polymerase core.

MATERIALS AND METHODS

Reagents, DNAs, Enzymes, and Replication Proteins—NTPs and dNTPs were from Pharmacia Biotech Inc. [α-32P]dATP was from Amersham. Alkaline phosphatase was from Boehringer Mannheim. Single-stranded circular DNAs from bacteriophages ϕAY-7/M and ϕR229-A/33 were prepared as described previously (34). pBROTB form I DNA was prepared as described (35).

PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG were purified as described (36). DnaA and HU were purified by an unpublished procedure,

*2 This work was supported by National Institutes of Health Grants GM34557 (to K. J. M.) and GM36255 (to C. S. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: pol III HE, the DNA polymerase III holoenzyme; SSB, the single-stranded DNA-binding protein; TFII, tailed form II; ERI, early replication intermediate; kb, kilobase pair(s); nt, nucleotide(s).

2 D. Langley and K. J. Marians, unpublished data.

3 M. Olson, J. Carter, H. G. Dallmann, and C. S. McHenry, unpublished data.
TFII DNA, 3.2 nM DnaB, 56 nM DnaC, 240 nM DnaG (or as indicated), 28 nM DnaT, 2.5 nM PriA, 2.5 nM PriB, 2.5 nM PriC, and the pol III core, αε or α as indicated and all other HE subunits at 28 nM, were preincubated at 30 °C for 2 min. NTPs were added to final concentrations of 1 mM ATP, 200 μM GTP, 200 μM CTP, and 200 μM UTP, and dNTPs to 40 μM. The reaction was incubated for 2 min at 30 °C. [α-32P]dATP (2000–4000 cpm/pmol) was added to the reaction mixture and the incubation was continued at 30 °C for an additional 10 min. DNA synthesis was quenched by addition of EDTA to 40 mM. Total DNA synthesis was determined by assaying an aliquot of the reaction mixture for acid insoluble radioactivity. The reaction mixtures were treated with alkaline phosphatase (3 units) at 37 °C for 45 min and the DNA products analyzed by alkaline gel electrophoresis as described (31). The αε-type primosomal proteins were used rather than just DnaB, DnaC, and DnaG because the former group of proteins assembles replication forks about 15-fold more efficiently than the latter (41).

Determination of Coupling of Leading- and Lagging-strand Synthesis—Standard rolling circle reaction mixtures containing core, αε or α as indicated were incubated for 2 min at 30 °C in the absence of label to establish active replication forks. Aliquots (1 μl) were then transferred to a prewarmed dilution reaction mixture (90 μl) containing all buffer components, NTPs, dNTPs, [α-32P]dATP, SSB, and primase at their standard concentrations, but that lacked DNA template, all other HE subunits, and the preprimosomal proteins (PriA, PriB, PriC, DnaT, DnaC, DnaG, and Dna). The incubation was continued at 30 °C for 10 min and terminated by the addition of EDTA. As a control for Okazaki fragment size, the original reaction mixture was incubated at 30 °C for 10 min in the presence of [α-32P]dATP and in the absence of any diluent. Reactions were then processed and analyzed as described above.

Determination of Replication Fork Processivity—Standard rolling circle reaction mixtures were assembled either in the presence of core, αε or α as indicated. The effect of the anti-β antibody (42) on initiation was assessed by including it in the reaction mixture from the start, before the ATP concentration was raised and before the dNTPs and other NTPs were added. The effect of the antibody on elongation was determined by adding the antibody along with the [α-32P]dATP 2 min after the reaction had been initiated. Reactions were then processed and analyzed as described above.

Determination of Replication Fork Rates—Standard rolling circle replication reactions containing core, αε or α were increased in size 4-fold. Reaction mixtures were incubated for 6 min at 30 °C after the addition of [α-32P]dATP. 5-Methyl-dCTP was then added to a final concentration of 0.4 mM and aliquots (7 μl) were withdrawn every 10 s for the next minute. Each aliquot was mixed with 1 μl of 20 μM dCTP to terminate the elongation reaction. Aliquots were then heated at 68 °C for 10 min and then treated with 10 units each of the AluI, HaeIII, HpaI, and HpaII restriction endonucleases for 2 h at 37 °C. DNA products were analyzed by electron microscopy through alkaline-agarose gels (20 × 25 × 0.5 cm) at 40 V for 48 h. The ecoli buffer was changed once after 24 h.

Determination of Replication Fork Stability—oriC DNA replication was reconstituted as described by Hiasa and Marinas (43) using pBROTB DNA as the template in the presence of DnaA, DnaB, DnaC, DnaG, SSB, HU, and the pol III HE reconstituted with either core, αε or α as indicated. Replication reactions (75 μl) were initiated in the absence of any topoisomerase. After a 2-min incubation, [α-32P]dATP was added and the incubation continued for 1 min. Under these conditions only an early replication intermediate (ERI) is formed. Subsequent nascent chain elongation requires the release of the accumulated topological constraint. The label was chased by the addition of an 100-fold excess of cold dATP (time 0) and the incubation continued. Aliquots (15 μl) were removed at the indicated times, mixed with the Smal restriction endonuclease (20 units), and incubated an additional 10 min. DNA products were then analyzed by electron microscopy through denaturing alkaline-agarose gels as described (45).

RESULTS

Okazaki Fragment Synthesis Is Modulated Properly at Replication Forks Containing only αε—Incubation of the TFII DNA template, SSB, the primosomal proteins, and the pol III HE generates replication forks that support rolling circle DNA replication. These forks produce multigenome length double-stranded tails that are composed of a long, continuous leading strand and short (about 2 kb) Okazaki fragments (31). We have demonstrated that the forks formed in vitro possess many of the characteristics of bona fide E. coli replication forks. They are highly processive, synthesizing leading strands in excess of 0.5 megabase in length (31), move at rates comparable to that of the fork in vivo (31, 41), exhibit coupled leading- and lagging-strand DNA synthesis (20, 44), and regulate the size of the Okazaki fragments produced in response to various reaction parameters (31, 44–47). To examine the roles of the three subunits of the polymerase core at the replication fork, we therefore assessed the ability to form replication forks with αε and α alone, as well as the characteristic properties of the forks formed.

HE reconstituted with αε, αε or α was titrated in rolling circle replication reactions in the presence of the TFII DNA template, SSB, and the primosomal proteins. Neither ε nor θ were required to form replication forks capable of producing long leading strands and short Okazaki fragments (Fig. 1). The lagging-strand products were of typical size, between 1 and 2 kb in length. We did note that a higher concentration of HE containing only αε was required to produce active replication forks. This probably reflects either a decrease in affinity for the template for αε compared with αε or a decrease in affinity of one of the protein-protein interactions required to establish a replication fork. This is consistent with the lower level of DNA synthesis observed with αε alone, presumably reflecting a lower efficiency of initiation.

Okazaki fragment size is governed by a transient protein-protein interaction between primase and DnaB at the replication fork (33, 46). DnaG acts distributively with respect to the cycle of lagging-strand synthesis, loading onto the template via interaction with the helicase, synthesizing a primer, and then dissociating from the fork to be replaced by another molecule from solution for the next round of primer synthesis (32). Thus, Okazaki fragment size is inversely proportional to both the
primase concentration (31, 46) and the affinity of the protein—protein interaction between primase and DnaB (33).

Replication forks formed with \( \alpha \varepsilon \theta, \alpha \varepsilon, \) or \( \alpha \) responded in an identical fashion to primase concentration (Fig. 2). This indicated that neither \( \varepsilon \) nor \( \theta \) were required for the interaction between primase and the replication fork and that their absence from the fork in no way compromised cycling of the lagging-strand polymerase from the just completed Okazaki fragment to the new primer. This argues that neither dissociation of the lagging-strand polymerase from the replication fork in no way compromised cycling of the polymerase between primase and the replication fork and that their absence from the fork in no way compromised cycling of the lagging-strand polymerase from the just completed Okazaki fragment to the new primer. This argues that neither dissociation of the lagging-strand polymerase from the replication fork in no way compromised cycling of the polymerase between primase and the replication fork, and that their absence from the fork in no way compromised cycling of the polymerase between primase and the replication fork and that their absence from the fork in no way compromised cycling of the polymerase between primase and the replication fork.

Replication Forks Containing Only \( \alpha \) Are Processive, Move at High Rates, and Elaborate Coupled Leading- and Lagging-strand Synthesis—The replication forks that form at oriC are extraordinarily processive. Each fork presumptively synthesizes a leading strand of about \( 2.3 \times 10^6 \) nt in length in one polymerase binding event. We have developed a protocol that allows us to test replication fork processivity (41) that makes use of the observation that once formed in an elongation complex with \( \alpha \), \( \beta \) is no longer accessible to antibody (42). Thus, replication forks that are processive are resistant to inhibition by anti-\( \beta \) antibody.

Rolling circle replication reactions containing \( \alpha \varepsilon \theta, \alpha \varepsilon, \) or \( \alpha \) received the anti-\( \beta \) antibody either 3 min before initiation (I in Fig. 3) or 3 min after initiation (E in Fig. 3). When added before initiation, all DNA replication was inhibited with any combination of core subunits, as would be expected because the replication fork cannot form without free \( \beta \) (Fig. 3). On the other hand, when the anti-\( \beta \) antibody was added after replication forks had formed, only lagging-strand synthesis was inhibited, long leading strands that could not enter the alkaline-agarose gel were still formed for each combination of core subunit (Fig. 3). Inhibition of lagging-strand synthesis was expected because the synthesis of each new Okazaki fragment requires a new \( \beta \) dimer (31, 46). Note that the Okazaki fragments synthesized in this experiment are much larger than the typical ones (e.g. in Figs. 1 and 2). This was because the concentration of \( \beta \) was lowered in this experiment to observe the effect of the antibody. Because \( \beta \) is used stoichiometrically during lagging-strand synthesis, Okazaki fragment size is inversely related to its concentration (31, 48). These experiments demonstrate that replication fork processivity is unaffected by the absence of either the \( \varepsilon \) or \( \theta \) subunits.

The E. coli replication fork moves at about 1000 nt/s at 37 °C. Replication fork speed in the rolling circle system has been measured at 600–800 nt/s at 30 °C (31, 41). We therefore investigated whether the \( \varepsilon \) or \( \theta \) subunits had any affect on replication fork speed.

The technique used measures the speed of established replication forks in the following manner (31), rolling circle replication reactions are initiated under normal conditions in the presence of \([\alpha^{-32P}]dATP\). After 6 min of incubation, when significant initiation of replication has occurred, 5-methyl-dCTP is added in 10-fold excess over dCTP and aliquots are taken every 10 s for 1 min. The reaction is then terminated by adding a 60-fold excess of ddTTP over dTTP. This generates long, labeled, leading strands that are unmethylated except in the regions synthesized during the last minute of synthesis. The unmethylated regions are removed by digestion with restric-
Replication Fork Processivity

Fig. 4. Replication forks formed with \( \alpha \varepsilon \theta \), \( \alpha \varepsilon \), or \( \alpha \) move at identical rates. Replication fork rates were determined as described under "Material and Methods". The time indicated refers to the time after the addition of 5-methyl-dCTP to the reaction mixture.

The analysis of replication fork speed for forks containing \( \alpha \varepsilon \theta \), \( \alpha \varepsilon \), or \( \alpha \) is shown in Fig. 4. The size of the leading strands in all three cases is nearly identical for each replication fork at each time point during the 1-min sampling period. This indicates that the rate of replication fork progression in each case was identical. The actual values, calculated using PhosphorImager traces of each lane to locate the trailing edge of the smear, for the forks containing core, \( \alpha \varepsilon \), and \( \alpha \) were 660, 656, and 654 nt/s, respectively.

The analysis of replication fork speed for forks containing \( \alpha \varepsilon \theta \), \( \alpha \varepsilon \), or \( \alpha \) is shown in Fig. 4. The size of the leading strands in all three cases is nearly identical for each replication fork at each time point during the 1-min sampling period. This indicates that the rate of replication fork progression in each case was identical. The actual values, calculated using PhosphorImager traces of each lane to locate the trailing edge of the smear, for the forks containing core, \( \alpha \varepsilon \), and \( \alpha \) were 660, 656, and 654 nt/s, respectively.

Replication Fork Processivity

Fig. 5. Replication forks formed with either \( \alpha \varepsilon \theta \), \( \alpha \varepsilon \), or \( \alpha \) exhibit coupled leading- and lagging-strand DNA synthesis. Standard rolling circle replication reaction mixtures containing HE reconstituted with \( \alpha \varepsilon \theta \) (lanes 1 and 2), \( \alpha \varepsilon \) (lanes 3 and 4), or \( \alpha \) (lanes 5 and 6) were diluted 90-fold as described under "Materials and Methods." B and A refer to DNA products made before and after dilution, respectively.

In the polymerase associating with the primer, or, as we have observed (20), the entire fork would fall apart and synthesis would not be observed at all.

Fig. 5 shows that lagging-strand synthesis is preserved when replication forks containing core, \( \alpha \varepsilon \), or \( \alpha \) are diluted 90-fold into reaction mixtures that contain only SSB, DnaG, and \( \beta \) at their original concentrations, all other protein components were omitted from the dilution reaction mixture. The concentrations of SSB, DnaG, and \( \beta \) must be maintained after dilution because each of these proteins acts distributively during multiple cycles of lagging-strand synthesis (31). These results are identical to those we have observed previously with bona fide HE purified from bulk \( E. coli \) (20, 44). As we have remarked before (20), we consistently observe a slight shift in Okazaki fragment size after dilution that we believe is attributable to the large excess of SSB over active template in the dilution reaction mixture. Thus, neither \( \varepsilon \) nor \( \theta \) are required for coupling of the leading- and lagging-strand polymerases at the replication fork.

Replication Forks Formed with \( \alpha \varepsilon \theta \), \( \alpha \varepsilon \), or \( \alpha \) Exhibit Identical Stabilities—The presence or absence of a 3' → 5' proofreading exo-nuclease function might only affect the stability of the polymerases when the fork was paused. Thus, we considered that we might not observe any differences between replication forks in the presence and absence of \( \varepsilon \) when they were moving, as they were in all the experiments described above. Because it is difficult to pause replication forks in the rolling circle replication assay, we used the \( oriC \) replication system, where forks can be paused as a result of accumulated positive overwindings in the template DNA (51), to investigate this issue.

This protocol exploits the fact that \( oriC \) replication will initiate on a superhelical plasmid DNA template in the absence of a topoisomerase. Replication proceeds until excess positive supercoils accumulate, causing the replication forks to pause, resulting in the formation of an ERI where the nascent leading strands are about 600 nt in length. Replication fork progression is resumed if the accumulated topological constraint is relieved...
the same in all cases. Replication forks containing core, αε, or ε displayed identical stabilities, decaying with essentially the same rates (Fig. 6B). Thus, the presence of ε, even under conditions where the exonuclease would be expected to be active, does not have an affect on replication fork stability.

DISCUSSION

The replication fork is a complex structure where upward of 30 protomers combine to execute ordered, semi-conservative DNA replication in a rapid and highly efficient fashion. Understanding the role of the polypeptides at the fork is the key to understanding how the replisome functions. We have been using a rolling circle DNA replication system reconstituted with purified proteins in an effort to contribute to this understanding. The availability of all the protein components in highly purified form has allowed an analysis of the replication fork functions that are disrupted when various polypeptides are omitted from replication fork assembly. Such analyses revealed, for example, the requirement for a protein-protein interaction between the σ subunit of the HE and DnaB that literally defined the replisome, cementing the polymerase and the helicase together, enabling rapid replication fork movement (28), and determining which of the two polymerase cores will be the leading-strand polymerase (29, 30).

In this report we have considered the contributions of the three subunits of the polymerase core to replication fork function. α, ε, and θ purify as a tight 1:1:1 complex when polymerase activity is scored by, e.g. using nicked salmon sperm DNA as the template (11). When processive DNA synthesis is used as the assay, the core is isolated as a component of the HE (8). θ is the product of holE (12, 52), which can be disrupted in E. coli without any apparent affect (53). Biochemical analyses have also failed to attribute a catalytic activity to θ, ε is the proof-reading 3′ → 5′ exonuclease (10) encoded by dnaQ (54). It can form a tight complex with both α and θ, apparently forming the bridge between them in the core (12). Strains deficient in ε show the expected mutator effect (55). α is the polymerase subunit (9), encoded by dnaH (56) and is, of course, essential for viability.

Because these three proteins form such a tight complex, it is expected that they are present at the replication fork. On one level, the contribution of ε is obvious. At a different level, we have investigated whether ε and θ played additional roles that affected replication fork function directly. We have assessed the distinguishing characteristics of replication forks that contained αθ, αε, or only α. We found that replication forks that contained only α performed in a fashion indistinguishable from those containing the complete core. Thus, neither ε nor θ were required to either maintain high rates of processive replication fork movement, to couple the leading- and lagging-strand polymerases, or to ensure proper recycling of the lagging-strand polymerase from the just completed Okazaki fragment to the new primer terminus. This makes it unlikely that these subunits are required to maintain the structural integrity of the replisome. Replication fork assembly did require higher concentrations of α than αε. This reduced efficiency suggests that ε may facilitate the interaction of α with another protein at the replication fork.

In a study examining processivity of αθ, αε, and α on primed single-stranded phage DNAs in the presence of β and the γ complex, Studwell-Vaughan and O’Donnell (12) concluded that αε, while maintaining a reasonable processivity in the range of 1–3 kb, was still significantly less processive than the combination of αε and suggested that highly processive DNA synthesis by the HE was contingent on the exonuclease subunit. Kim and McHenry (14) did not observe this difference when they used γ complex to load β onto the DNA and an anti-β

---

**Figure 6.** Replication forks formed with αθ, αε, or α exhibit identical stabilities. A, ERIs were formed and labeled in standard oriC DNA replication reactions containing HE reconstituted with αθ lanes 1–5), αε (lanes 6–10), or α (lanes 11–15) as described under “Materials and Methods.” Excess cold ATP was added at 0 time, aliquots were withdrawn at the indicated times, the Smal restriction endonuclease (20 units) was then added to each aliquot, and the aliquots were incubated for an additional 10 min. The reactions were processed and analyzed as described under “Materials and Methods.” B, the relative extent of elongation of ERI to linear DNA is plotted as a function of time of incubation of the paused ERI.

---

by either the addition of a topoisomerase or by breaking the phosphodiester backbone of the template. The use of a restriction endonuclease to relieve topological constraint results in a replication fork run-off on the linear template (6.0 kb).

To assess replication fork stability, we actually measured decay of the capacity of paused forks in the ERI to resume DNA replication. ERIs were formed containing αθ, αε, or α in the presence of [α-32P]dATP. An 100-fold excess of nonradioactive ATP was added (time 0) and aliquots were removed at the indicated times. Aliquots were processed by the addition of the Smal restriction enzyme to linearize the template and an additional 10-min incubation to permit elongation by active replication forks. When analyzed by native agarose gel electrophoresis (Fig. 6A) two bands are therefore observed, ERI that has not been elongated and linear DNA, which represents the product of active replication forks. The stability of paused replication forks in the ERI is then measured as the fraction of ERI that can be converted to linear DNA as a function of time (Fig. 6B).

We do not, as yet, know the reason that the forks decay and there are, of course, many possibilities. However, for the purpose of this assay, we assume that the mechanism of decay is
antibody challenge similar to the one described in this report to test processivity. This latter report did note that HE reconstituted with only α synthesized DNA at about one-fifth the rate of HE reconstituted with α and αβ. Thus, the results observed at bona fide replication forks are somewhat different from each of these reports, underscoring the importance of assessing function in the proper context. Presumably, the major difference is that the α-DnaB interaction that occurs at replication forks is the overriding factor in the determination of fork rate and processivity.

However, a structural role for δ at the replication fork under certain conditions cannot be completely ruled out. dnaQ disruptions in Salmonella typhimurium display two phenotypes, a mutator defect and a slow growth defect (57). Spontaneous suppressor mutations of the slow growth phenotype that have been mapped to δ arise very rapidly in these strains. The purified suppressor polymerase was shown to have 3–5-fold higher activity than the wild-type polymerase (58). This suggests that α activates δ in vivo. It is interesting to consider that this apparent difference may reflect the basic difference between the rolling circle replication system and replication forks on the chromosome of E. coli. That is, the in vitro system proceeds in an unimpeded fashion around the template, whereas in the cell, replication forks encounter all sorts of protein roadblocks on the DNA as well as damaged bases in the template. It may be that under these circumstances δ is required for stability of the fork. This possibility awaits further investigation.

REFERENCES

1. Marians, K. J. (1992) Annu. Rev. Biochem. 61, 673–719
2. LeBowitz, J. H., and McMacken, R. (1986) J. Biol. Chem. 261, 4738–4748
3. Kaguni, J. M., and Kornberg, A. (1984) Cell 38, 183–190
4. Bouché, J. P., Zechel, K., and Kornberg, A. (1975) J. Biol. Chem. 250, 5995–6001
5. Kogoma, T. (1996) Cell 85, 625–627
6. Ng, J. Y., and Marians, K. J. (1996) J. Biol. Chem. 271, 15642–15648
7. Ng, J. Y., and Marians, K. J. (1996) J. Biol. Chem. 271, 15649–15655
8. Maki, H., Maki, S., and Kornberg, A. (1988) J. Biol. Chem. 263, 6570–6578
9. Welch, M. M., and McHenry, C. S. (1982) J. Bacteriol. 152, 351–356
10. Scherzmann, R. H., and Echols, H. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7174–7178
11. McHenry, C. S., and Crow, W. (1979) J. Biol. Chem. 254, 1748–1753
12. Studwell-Vaughan, P. S., and O'Donnell, M. (1993) J. Biol. Chem. 268, 11785–11791
13. Maki, H., and Kornberg, A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4389–4392
14. Kim, D. R., and McHenry, C. S. (1996) J. Biol. Chem. 271, 20681–20689
15. Studwell, P. S., and O'Donnell, M. (1999) J. Biol. Chem. 274, 1171–1178
16. Dallmann, H. G., and McHenry, C. S. (1995) J. Biol. Chem. 270, 29563–29569
17. Onrust, R., Finkbeiner, J., Turner, J., Naktinis, V., and O'Donnell, M. (1985) J. Biol. Chem. 270, 13386–13377
18. McHenry, C. S. (1982) J. Biol. Chem. 257, 2657–2663
19. Stukenberg, P. T., and O'Donnell, M. (1995) J. Biol. Chem. 270, 13384–13391
20. Kim, S., Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1996) J. Biol. Chem. 271, 21406–21412
21. McHenry, C. S., and Kornberg, A. (1977) J. Biol. Chem. 252, 6478–6484
22. Stukenberg, P. T., Turner, J., and O'Donnell, M. (1996) J. Biol. Chem. 271, 21328–21336
23. Kim, S., Dallmann, H. G., McHenry, C. S., and Kornberg, A. (1977) J. Biol. Chem. 252, 6478–6484
24. O'Donnell, M. (1987) J. Biol. Chem. 262, 16534–16536
25. Dallmann, H. G., and McHenry, C. S. (1993) J. Biol. Chem. 268, 11328–11334
26. Kong, K., Ohrn, R., Onrust, R., and Kurjian, J. (1992) Cell 69, 425–437
27. Kim, S., Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1996) Cell 84, 643–650
28. Yuzhakov, A., Turner, J., and O'Donnell, M. (1986) Cell 66, 877–886
29. Wu, C. A., Zechnher, E. L., and Marians, K. J. (1992) J. Biol. Chem. 267, 2030–4044
30. Tsug, K., Pong, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 4675–4682
31. Tsug, K., and Marians, K. J. (1996) J. Biol. Chem. 271, 21398–21405
32. Model, P., and Zinder, N. (1974) J. Biol. Chem. 253, 231–251
33. Marians, K. J., Soeller, W., and Zupuran, L. (1982) J. Biol. Chem. 257, 5656–5662
34. Marians, K. J. (1995) Methods Enzymol. 262, 507–521
35. Johanson, K. O., Haynes, T. E., and McHenry, C. S. (1986) J. Biol. Chem. 261, 11460–11465
36. Dallmann, H. G., Thimmig, R. L., and McHenry, C. S. (1995) J. Biol. Chem. 270, 29555–29562
37. Olson, M. W., Dallmann, H. G., and McHenry, C. S. (1995) J. Biol. Chem. 270, 29570–29577
38. Minden, J. S., and Marians, K. J. (1985) J. Biol. Chem. 260, 9316–9325
39. Mok, M., and Marians, K. J. (1987) J. Biol. Chem. 262, 16644–16654
40. Johanson, K. O., and McHenry, C. S. (1982) J. Biol. Chem. 257, 12310–12315
41. Hiisa, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 5655–5663
42. Wu, C. A., Zechnher, E. L., Hughes, J., A. J., Fraden, M. A., McHenry, C. S., and Marians, K. J. (1992) J. Biol. Chem. 267, 4604–4703
43. Zechnher, E. L., Wu, C. A., and Marians, K. J. (1992) J. Biol. Chem. 267, 4045–4083
44. Wu, C. A., Zechnher, E. L., Reems, J. A., McHenry, C. S., and Marians, K. J. (1992) J. Biol. Chem. 267, 4074–4083
45. Stukenberg, P. T., Turner, J., and O'Donnell, M. (1994) J. Biol. Chem. 269, 20681–20689
46. Stukenberg, P. T., and O'Donnell, M. (1999) Nucleic Acids Res. 27, 3281–3286
47. Carter, J. L., Franden, M. A., Aebersold, R., Kim, D. R., and McHenry, C. S. (1993) Nucleic Acids Res. 21, 3281–3286
48. Slater, S. C., Lifsics, M. R., O'Donnell, M., and Maurer, R. (1994) J. Bacteriol. 176, 815–821
49. Scheuermann, R. H., Tam, S. M., Burgers, P. J. M., Lu, C., and Echols, H. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7085–7089
50. Stukenberg, P. T., Turner, J., and O'Donnell, M. (1994) Cell 78, 877–887
51. Dallmann, H. G., and McHenry, C. S. (1993) J. Bacteriol. 175, 1171–1178
52. Lifsics, M. R., and Lancy, E. D., and Maurer, R. (1999) J. Bacteriol. 171, 5572–5580
53. Lifsics, M. R., Lancy, E. D., and Maurer, R. (1992) J. Bacteriol. 174, 6965–6973