Two Modes of PriA Binding to DNA*

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The role of PriA, required for the assembly of the φX174-type primosome on DNA, in cellular DNA replication has been unclear since its discovery. Recent evidence, based on the phenotypes of strains carrying priA null mutations, has led to proposals that the primosome assembly activity of PriA was required to load replication forks at intermediates such as D loops during homologous recombination. McGlynn et al. (McGlynn, P., Al-Deib, A. A., Liu, J., Marians, K. J., and Lloyd, R. G. (1997) J. Mol. Biol. 270, 212–221) demonstrated that PriA could, in fact, bind D loops. We show here that there are two modes of stable binding of PriA to DNA. One mode, in which the enzyme binds 3′ → 5′ DNA helicase activity of PriA. The D loop DNA binding activity of PriA can be accounted for by the second mode, where the enzyme binds bent DNA at three strand junctions.

PriA, a 3′ → 5′ DNA helicase (1, 2), was discovered originally because of its requirement during synthesis of the complementar- istic strand of φX174 viral DNA during the initial stage of DNA replication in the life cycle of the bacteriophage (3, 4). Biochemical analyses showed that PriA was required for the assembly of the primosome, a multienzyme replication machine that, once formed at a specific structure on φX174 single-stranded circular (ss(c)) DNA, could translocate along the DNA occasionally synthesizing short oligonucleotide primers that could be used to initiate synthesis of the complementary strand (5).

Subsequent studies revealed that the primosome could provide both the DNA unwinding function, via the action of DnaB (6), and the priming function, via the action of DnaG (7, 8), needed for replication fork propagation. Primosome assembly required, in addition to PriA, six other proteins, PriB, PriC, DnaT, DnaB, DnaC, and DnaG. As a result of genetic evidence and biochemical studies of oriC DNA replication, the roles of DnaB, DnaG, and DnaC in chromosomal replication have long been clear (9). However, those of PriA, PriB, PriC, and DnaT, which are not required for replication from oriC (10), have been problematic. Recent genetic studies have now provided a likely role for these proteins during cell growth.

Escherichia coli strains carrying null mutations in priA have a complex set of phenotypes that includes reduced viability (11–13), chronic induction of the SOS response (12), rich media sensitivity (14), reduced ability to undergo homologous recombination (15, 16), sensitivity to UV irradiation (11, 15, 16), and defective double-strand break repair (16), induced stable DNA replication (14), and constitutive stable DNA replication (14). All of these phenotypes could be suppressed when a priA allele encoding a mutant PriA protein no longer capable of acting as a DNA helicase, but still capable of catalyzing primosome assembly (17), was provided in trans (14–17). This observation, and the requirement for recombination proteins during double-strand break repair, and both induced and constitutive stable DNA replication, led to the proposal that the cellular function of PriA was to catalyze the assembly of replication forks at recombination intermediates such as D loops (15, 16). This was consistent with our initial proposal, based on the manifestation of chronic SOS induction in the absence of exogenous DNA damage in priA null strains, that PriA-directed replication fork loading was required under conditions of normal cell growth to rescue replication forks that had initiated at oriC, but had stalled before they could complete synthesis of the genome (18).

In support of this model for PriA action, McGlynn et al. (19) demonstrated that the enzyme could bind D loop DNA. Using gel shift analysis, we show here that PriA can bind to two types of DNA structures: duplex DNAs with 3′ → 5′-single-stranded extensions and bent DNA at three stranded junctions. The latter activity accounts for the high affinity binding of PriA to D loop DNA. Interestingly, the inability of PriA to bind bubble structures derives from the disposition of the single strands in the bubble region. In the accompanying report (20), we describe PriA-catalyzed primosome assembly at D loops.

MATERIALS AND METHODS

Oligonucleotides—The DNA sequences of all oligonucleotides (oligos) used is shown in Table I.

Assembly of DNA-binding Substrates—Oligos were 5′-end-labeled using [γ-32P]ATP and bacteriophage T4 polynucleotide kinase. Unless noted otherwise, the top strand (oligo 1 or 1B, Table I) was labeled in all DNA-binding substrates used. Oligos annealed to give the various substrates are listed in Table II. Annealing reaction mixtures (20 μl) covered with 25 μl of mineral oil in 0.5 ml of thin-walled tubes containing the 5′-32P-labeled oligo at 1 μM, all unlabeled oligos at 3 μM, 10 μM Tris-HCl (pH 7.5 at 4 °C), 7 mM MgCl2, and 200 mM NaCl were heated at 95 °C for 10 min, transferred directly to 65 °C and held at that temperature for 1 h, slow-cooled to 25 °C over a period of 2 h, and then chilled on ice. Substrates were then purified by electrophoresis through 6% polyacrylamide (29:1, acrylamide:bisacrylamide) gels at 30 mA for 1 h using 50 mM Tris, 40 mM boric acid, 1 mM EDTA as the electrophoresis buffer. Substrates were eluted from the gel by crushing the gel slice in 500 μl NH4OAc, 10 mM MgOAc, 1 mM EDTA, and incubating overnight at 4 °C. The slurry was then filtered through Spin-X columns (Costar), the DNA recovered by ethanol precipitation and resuspended in 10 mM Tris-HCl (pH 7.5 at 4 °C), 5 mM MgCl2.

DNA Binding Assay—PriA was purified as described by Marians (21). DNA-binding reaction mixtures (15 μl) containing 50 mM Tris-HCl (pH 8.3 at 23 °C), 10 mM MgCl2, 10 mM dithiothreitol, 1 μg/ml bovine serum albumin, 0.2% Triton X-100, 1 mM DNA substrate, and the indicated concentrations of PriA were incubated at 30 °C for 10 min and then loaded directly onto 10 × 13 × 0.12-cm 6% polyacrylamide (90:1, acrylamide:bisacrylamide) gels. Electrophoresis, with constant recirculation of the buffer, was at 13 mA for 3 h at 4 °C using 6 mM Tris-HCl (pH 7.8), 5 mM NaOAc, 2 mM MgOAc, and 0.1 mM EDTA as the electrophoresis buffer. The gels were dried, exposed to PhosphorImager

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† The abbreviations used are: ss(c), single-stranded (circular); oligo, oligonucleotide; nt, nucleotides; SSB, single-stranded binding protein.
screens, and autoradiographed. Quantitation was by the use of a Fuji PhosphorImager. Binding constants were determined using the Hill equation as described (22).

**RESULTS**

**PriA Binds D Loops**—McGlynn et al. (19), demonstrated that PriA could bind to D loop DNA. In order to assess which features of a D loop were recognized by PriA, we constructed a nested series of DNA-binding substrates that modeled different features of one particular D loop DNA and tested their binding to the enzyme by gel mobility shift analysis. The two basic substrates used to show the specific affinity of PriA for D loops were a bubble DNA consisting of oligos 1 and 2L (Tables I and II) and a D loop DNA consisting of oligos 1, 2L, and 5S (Tables I and II). These DNA-binding substrates were 82 nucleotides (nt) long with a central noncomplementary bubble region of 42 nt flanked by 23 and 17 nt of duplex DNA to the left and right, respectively, as drawn in the figures. The 3'/invading strand (oligo 5S) was 42 nt in length and was complementary to oligo 1. For purposes of clarity, in all diagrams oligo 1 or 1B is presented as the top 5'-3' strand.

**TABLE I**

| Oligonucleotide | Sense | Length | Sequence (5’→3’) |
|-----------------|-------|--------|-----------------|
| dlp1 Top        | 82    | AATTCGGTTGACGTTCTAGTG | |
| dlp1B Top       | 59    | AGCCGATACGGTATAAGGGCT | |
| dlp2L Bottom    | 82    | TACGTTGTCGGCGTGGCAGC | |
| dlp2L-20 Bottom | 62    | ATTTGGGTTGACGTTCTAGTG | |
| dlp2-38 Bottom  | 44    | CGTTACCCTACCCCCATAAAA | |
| dlp3 Top        | 21    | AGCCCTTATCCGATTTGGCCT | |
| dlp3L Bottom    | 38    | CGCGCCGAGACTCAATTTGAC | |
| dlp4-4 Bottom   | 29    | ACTCATTGACGCTTATCCGT | |
| dlp5 Invading   | 54    | GCAGACTATTGGAATGGTCGTCGCGTGCT | |
| dlp5S Invading  | 42    | TGAATTGTCGGCGGCGGCGGCGT | |
| dlp11B Bottom   | 38    | TACGTTGTCGGCGGCGGCGT | |
| dlp11B-3 Bottom | 35    | TACGTTGTCGGCGGCGGCGT | |
| dlp12 Bottom    | 23    | AGCCCTTATCCGATTTGGCGGTT | |
| dlp15 Bottom    | 59    | TACGTTGTCGGCGGCGGCGT | |
| dlp15-24 Bottom | 35    | TACGTTGTCGGCGGCGGCGT | |
| dlp16 Bottom    | 17    | TACGTTGTCGGCGGCGT | |
| dlpB Top        | 18    | AAATGAGTCTGCGGCGGCGGCGT | |
| dlpC Bottom     | 56    | TACGTTGTCGGCGGCGGCGGCGT | |
| dlpC-10 Bottom  | 46    | TACGTTGTCGGCGGCGGCGGCGT | |
| dlpD Top        | 18    | CGCGCCGAGACTCATT | |

**FIG. 1. PriA binds specifically to D loop DNA.** PriA binding to the bubble and D loop substrates (Table II) was measured by gel mobility shift analysis as described under “Materials and Methods.” An autoradiograph of the gels shown in panel A and the quantitation of the data is shown in panel B.

Even though this difference in and of itself suggested that the D loop was in the form of the structure diagramed, we confirmed the structure of the D loop by: (i) demonstrating the presence of distinct electrophoretic mobilities for each of the oligo combinations involved (data not shown), (ii) demonstrating that the invading strand in the D loop could be elongated by DNA polymerase I for the predicted number of nucleotides (Fig. 2B), (iii) demonstrating that all regions predicted to be single-stranded in both the bubble and the D loop were sensitive to cleavage by S1 nuclease, but not to cleavage by DNase I, and (iv) demonstrating that all regions in both the bubble and D loop predicted to be duplex were sensitive to cleavage by DNase I, but not to cleavage by S1 nuclease (see the accompanying report, Ref. 20).
Under the conditions of the assay, stable PriA binding could not be detected to either ssDNA or duplex DNA (Fig. 3). This suggested that the specific binding of PriA to the D loop DNA arose as a result of PriA recognition of the regions in the D loop where the three strands intersected. We therefore tested the binding of PriA to substrates that mimicked the junctions between single- and double-stranded DNAs in the bubble and D loop substrates.

**PriA Binds Duplex DNAs with 3'-Single-stranded Extensions**—PriA did not bind bubble DNA, this suggested that it should not bind duplex DNA with either a 5’- or 3’-single-stranded extension. To assess if this were correct, a series of substrates were prepared that had a 35-nt long duplex region flanked by either 5'- or 3'-single-stranded tails of varying length. PriA bound with high affinity to duplexes with 3'-tails, whereas it did not bind to duplexes with 5'-tails at all (Fig. 4). High affinity binding ($K_d < 10$ nM) required a 3'-tail of 16 nt or greater in length. This finding was surprising because PriA could not bind to the bubble even though this structure contained 3'-single-stranded extensions from duplex DNA long enough to promote high affinity binding.

We considered that PriA might require a free 3’-single-stranded end to bind to DNA and that lack of binding to the bubble could be explained because there were no such ends in that structure. Accordingly, we assessed PriA binding to an 82-mer duplex DNA containing both a 21-nt (substrate 4) and a 42-nt long (substrate 5) single-stranded gap (Fig. 5, A and B). (The integrity of all structures composed of three oligos was confirmed by the existence of a unique electrophoretic mobility on native gels for the three-stranded structure compared with that of a two-stranded structure (Fig. 2A).) Both of these structures were bound with high affinity. Although we have not demonstrated this directly, the second, slower moving band shift observed with substrate 5 is most likely the result of the binding of two molecules of PriA to the substrate.

Because the data in Fig. 5, A and B, could not be used to explain the observed lack of PriA binding to the bubble, we considered that PriA might not be able to bind to gapped DNAs that also had a single-stranded 5’-tail on one side of the gap. However, PriA bound with high affinity to this type of a sub-

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**Fig. 2. Analysis of DNA-binding substrates.** A, formation of substrate 10 was analyzed by stepwise hybridization. Oligo 1B was labeled. Analysis was on a native 10% Tris borate polyacrylamide gel. All lanes shown were from the same gel, intervening lanes not relevant to this figure were removed. B, D loop 5 (Table II), composed of oligos 1, 2L, and 5, was used as a substrate for the Klenow polymerase in the presence of all four dNTPs at 15 °C. The invading strand was 5’-32P-end labeled. The products were analyzed by electrophoresis through a denaturing 12% Tris borate polyacrylamide gel containing 50% (w/v) urea. Extension of oligo 5 by 23 nt is observed, as predicted by its structure (Table I).

**Fig. 3. PriA does not bind either single-stranded or duplex DNA in the gel shift assay.** PriA binding was analyzed as described under “Materials and Methods” to substrate 1 (panel A) and oligo 1B (panel B). Some very weak binding of PriA to oligo 1B can be detected at the two highest concentrations of protein. This manifests as a decrease in intensity of the band of free substrate and the appearance of an indistinct smear above the position of free substrate.

**Fig. 4. PriA binds duplex DNA with a 3’-, but not a 5’-single-stranded extension.** PriA binding was analyzed as described under “Materials and Methods” to substrate 2 (panel A) and substrate 3 (panel B). C, effect of 3’-tail length on the binding to substrate 2 was quantitated as described under “Materials and Methods.” Oligo 1B was shortened from the 3’-end to give tails of the indicated length.
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FIG. 5. PriA does not require a free end to bind DNA. PriA binding was analyzed as described under "Materials and Methods" to substrate 4 (panel A), substrate 5 (panel B), and substrate 6 (panel C).

Thus, from the data presented, PriA should have bound the bubble DNA. We think it likely that this implies that the single strands in the bubble are not available for PriA binding. Whereas they are clearly not base paired (see the accompanying report, Ref. 20), they may still be wrapped about each other as in a tangle, rather than in a helical structure with a regular periodicity. We attempted to assess this by preparing bubble substrates where the two noncomplementary strands of the bubble were of different length. If wrapping of the two single strands about each other were a problem, it was possible that these substrates, where there should be 20-nt long single-stranded regions that are not wrapped around another strand and are adjacent to a duplex, might provide the region necessary for PriA binding. Using this substrate, some weak binding could be observed at high concentrations of PriA (Fig. 6). This manifested as a decrease in the amount of free substrate and the generation of a smear extending above the position of free substrate. This is indicative of very unstable binding. No distinct PriA-DNA complexes could be observed. Thus we conclude that in bubble structures, even though the noncomplementary strands are not base paired, they are also not in the same conformation as a single strand in a duplex DNA structure with a 3′-single-stranded tail.

PriA Binds Bent DNA at Three Strand Junctions—Even though PriA could bind duplex DNAs with 3′-tails and did not need a free 3′-end to manifest this activity, it could not bind a bubble. However, in a D loop, the duplex formed by the invading strand and the top strand is likely to be co-helical with the duplexes formed by the flanking sequences. This might prevent tangling of the single-stranded region of the bottom strand. This, in turn, could provide a site for PriA binding at the right-hand three-strand junction of the D loop. If this accounted for PriA binding to the D loop, we would expect to observe a preference for PriA binding to three strand junctions with 3′-single-stranded tails. Accordingly, we investigated this by assessing PriA binding to substrates 8 and 9 (Fig. 7).

As already noted, PriA bound the 5′- and 3′-tailed substrates with different affinity, it seemed unlikely that it was recognizing the nick in the substrate. The most likely feature responsible for PriA recognition was the bend in the DNA of the tailed oligo at the transition between the double- and single-stranded regions. The duplex regions formed by the three oligos of the substrate will be co-helical. This will force the tail to bend sharply out and away from the axis of the helix. This predicts that creation of a gap at the junction would relieve the necessity of a bend and decrease the affinity of PriA for the substrate. A series of substrates were therefore constructed with increasingly larger gaps at the three-strand junction. Two sets were examined that differed by the presence of a 5′-single-stranded tail at the junction.

As already noted, PriA bound a three-strand junction with a...
5'-single-stranded tail with high affinity (Fig. 8, B and C). Binding to such a junction in the absence of a tail (i.e., to a nick) was difficult to detect, having a dissociation constant over 10^5 greater than that of the three-strand junction (Fig. 8, A and C).

As the gap between the two bottom strands was enlarged, PriA binding to the structures with the 5'-tail became progressively worse, whereas binding to the structures without the tail actually improved to the point where it could be accurately measured. Nevertheless, binding to the structure containing an 8-nt gap without a tail was still quite poor. This is consistent with our observation that a minimum of 12 nt of ssDNA is required for stable binding of PriA (Fig. 4). We interpret the progressive loss of PriA binding affinity to three-stranded structures with tails as the gap between the two bottom strands increases as indicating that the enzyme is, in fact, recognizing the intersection of the three strands and that the primary recognition feature is the sharp bend made by the tail being forced out of the axis of the helix. This predicts that in a D loop, PriA will bind preferentially to the left-hand junction. This proved to be the case (see accompanying article, Ref. 20).

**PriA DNA Binding**

Recent genetic analyses of the phenotypes of *E. coli* strains carrying *priA* null alleles suggested that PriA was likely to participate in assembly of replication forks at recombination intermediates such as D loops (14–16). Accordingly, McGlynn et al. (19) demonstrated that PriA could bind D loop DNA. In this report, using a series of substrates composed of short oligonucleotides, we have investigated the underlying basis for the specificity of PriA DNA binding activity.

PriA shows remarkable discrimination in binding to D loop substrates with very high affinity but not to the corresponding bubble structure at all. PriA possesses a weak ssDNA binding activity as manifested by its ability to bind a ssDNA cellulose column at intermediate salt concentrations and by the fact that it is a ssDNA-dependent ATPase (23, 24). However, under the conditions of the gel shift assay used here, PriA binding could not be detected to either ssDNA or duplex DNA. Thus, binding to any of the three duplex regions of the substrate or to the displaced single strand could not account for PriA binding to the D loop. This suggested that PriA was recognizing some distinct structural feature of the D loop that was not found in the bubble.

Our analyses indicated that PriA could recognize and bind in a stable fashion to two types of three-strand junctions. PriA could bind to duplex DNA carrying a 3'-single-stranded extension with very high affinity, whereas it could not bind duplex DNA carrying a 5'-single-stranded extension at all. Stable binding could be detected with the 3'-extension when the single-stranded tail exceeded 12 nt in length and high affinity binding resulted when the tails were in excess of 16 nt in length. It seems likely that this mode of binding is a manifestation of the 3' → 5' DNA helicase activity of the enzyme.

Stable binding of PriA to duplex DNA with a 3'-tail could...
result from movement of the protein in the 3′ → 5′ direction along the single strand until it collides with the duplex. Unwinding would not occur because ATP is not present and the protein would thus be paused at the junction. In this scenario PriA would run off the 5′-tails, thus accounting for the lack of binding on those substrates. However, we have demonstrated that unidirectional movement of PriA along ssDNA required ATP hydrolysis (25). Thus, this is unlikely to be the reason for stable binding to the 3′-tailed duplex. Rather, as suggested by Lohman and Bjornson (26) in analyses of DNA helicase action, this mode of PriA binding probably results from an intrinsic ability of the enzyme to recognize such an orientation of duplex DNA and single-stranded tail. This ability to discriminate between 3′- and 5′-tails presumably contributes to the direction elaborated by the DNA helicase activity of the enzyme.

The second type of three-strand junction recognized by PriA is one where one strand at the junction forms a sharp bend. In the substrates used, PriA showed a 30-fold preference for junctions where the DNA bend resulted from a 5′-tail, rather than a 3′-tail. It was clear that in this mode of binding PriA was able to bind this substrate. This predicts that the protein should bind preferentially to the left-hand side of the D loop. This is demonstrated directly by DNA footprinting in the accompanying article (20).

We were unable to account definitively for the inability of PriA to bind the bubble structure. Given that binding to a duplex region with a 3′-single-stranded tail did not require a free 3′-end, PriA should have been able to bind this substrate. We suspect that even though the single-stranded regions in the bubble are clearly not base paired, because they are susceptible to S1 nuclease (see accompanying article, Ref. 20), they are tangled about each other as a result of the free rotation of the flanking duplex regions about their helical axes. This tangling presumably prevents access of PriA to the ssDNA.

Based on what we now know about PriA binding to DNA, can we account for the previous observations of specific binding of PriA to regions of DNA derived from bacteriophage and plasmid DNAs? Wickner and Hurwitz (23) showed initially that PriA possessed a specific DNA recognition function by the demonstration that φX174 ss(c) DNA was a 7–8-fold better effector of the PriA ATPase activity than φ1 ss(c) DNA. This paralleled the relative activity of the φX174-type primosome in DNA replication supported by these two DNAs as templates. Shlomai and Kornberg (27) showed that the discrimination shown by PriA toward these two DNAs in the ATPase assay increased to nearly 100-fold if the DNAs were coated by SSB. Specific stimulation of the PriA ATPase activity could be attributed to a 55-nt long DNA sequence located in the φX174 intergenic space (28). This region was resistant to digestion by exonuclease VII, suggesting that it possessed extensive secondary structure. Indeed, the DNA sequence could be folded on paper into a hairpin-like structure with a ΔG of −14 kcal/mol. This DNA region also served as the assembly site for the primosome (5).

Subsequently we demonstrated that there were two such sites on ColE1-type plasmid DNAs, one on each strand, that were active only when the DNA was in single-stranded form (29). Although only one of these was resistant to digestion by exonuclease VII, they both could be folded into hairpin structures on paper (30, 31). Mutational analysis demonstrated that single point mutations that inactivated the site destabilized the putative secondary structure by disrupting base pairs. Single revertant point mutations could be selected in some cases that restored the base pair by altering the partner nucleotide (31, 32). In addition, high concentrations of SSB could melt the sites and inactivate them (33). Thus, we concluded that PriA was recognizing some specific structural aspect of these DNAs and named them primosome assembly sites (34). Subsequently, primosome assembly site sequences were found on the F plasmid (35) and on several extrachromosomal DNAs (36, 37). Interestingly, such sites were never found on E. coli chromosomal DNA.

PriA recognition of primosome assembly site sequences may result from either mode of binding described here. Formation of a stable hairpin structure in ss(c) DNA in the presence of SSB may cause the DNA at the junction of the hairpin and SSB-coated DNA to bend sharply, providing a recognition feature. Alternatively, the formation of the hairpin may generate an SSB-free region at its base of sufficient size to allow PriA recognition of a 3′-single-stranded extension from a duplex DNA. Further investigation will be required to distinguish between these two possibilities.

REFERENCES
1. Lee, M. S., and Marians, K. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 24, 8345–8349
2. Lasken, R. S., and Kornberg, K. (1988) J. Biol. Chem. 263, 5512–5518
3. Wickner, S., and Hurwitz, J. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 4120–4124
4. Schekman, R., Weiner, J. H., Weiner, A., and Kornberg, A. (1975) J. Biol.
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Chem. 250, 5859–5865

5. Arai, K.-I., and Kornberg, A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 69–73
6. Bouché, J.-P., Zechel, K., and Kornberg, A. (1975) J. Biol. Chem. 250, 5995–6001
7. LeBowitz, J. H., and McMacken, R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 69–73
8. Wickner, S. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2815–2819
9. Marians, K. J. (1992) Annu. Rev. Biochem. 61, 673–719
10. Lee, E. H., and Kornberg, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3029–3032
11. Nurse, P., Zavitz, K. H., and Marians, K. J. (1991) J. Bacteriol. 173, 6686–6693
12. Sandler, S. J. (1996) Mol. Microbiol. 19, 871–880
13. Greenbaum, J. H., and Marians, K. J. (1995) Methods Enzymol. 262, 507–521
14. Yong, Y., and Romano, L. J. (1995) J. Biol. Chem. 270, 24509–24517
15. Wickner, S., and Hurwitz, J. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3342–3346
16. Shlomai, J., and Kornberg, A. (1980) J. Biol. Chem. 255, 6789–6793
17. Lee, M. S., and Marians, K. J. (1990) J. Biol. Chem. 265, 17078–17083
18. Lohman, T. M., and Bjornson, K. P. (1996) Annu. Rev. Biochem. 65, 169–214
19. Shlomai, J., and Kornberg, A. (1980) J. Biol. Chem. 255, 6794–6798
20. Shlomai, J., and Kornberg, A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 799–803
21. Zipursky, S. L., and Marians, K. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6521–6525
22. Marinas, K. J. (1985) CRC Crit. Rev. Biochem. 17, 153–215
23. Imber, R., Low, R., and Ray, D. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7132–7136
24. Nomura, N., Masai, H., Inuzuka, M., Miyazaki, C., Ohtsubo, E., Itoh, T., Sasamoto, S., Matsu, M., Ishizaki, R., and Arai, K.-I. (1991) Gene (Amst.) 108, 15–22
25. Masai, H., Nomura, N., Kubota, Y., and Arai, K.-I. (1990) J. Biol. Chem. 265, 15124–15133