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A euryarchaeal histone modulates strand displacement synthesis by replicative DNA polymerases

Fei Sun & Li Huang*

State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

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Euryarchaeota and Crenarchaeota, the two main lineages of the domain Archaea, encode different chromatin proteins and differ in the use of replicative DNA polymerases. Crenarchaea possess a single family B DNA polymerase (PolB), which is capable of strand displacement modulated by the chromatin proteins Cren7 and Sul7d. Euryarchaea have two distinct replicative DNA polymerases, PolB and PolD, a family D DNA polymerase. Here we characterized the strand displacement activities of PolB and PolD from the hyperthermophilic euryarchaeon *Pyrococcus furiosus* and investigated the influence of HPfA1, a homolog of eukaryotic histones from *P. furiosus*, on these activities. We showed that both PolB and PolD were efficient in strand displacement. HPfA1 inhibited DNA strand displacement by both DNA polymerases but exhibited little effect on the displacement of a RNA strand annealed to single-stranded template DNA. This is consistent with the finding that HPfA1 bound more tightly to double-stranded DNA than to a RNA∶DNA hybrid. Our results suggest that, although crenarchaea and euryarchaea differ in chromosomal packaging, they share similar mechanisms in modulating strand displacement by DNA polymerases during lagging strand DNA synthesis.

euryarchaea, PolB, PolD, strand displacement, archaeal histone

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INTRODUCTION

DNA polymerases are classified into different families based on their phylogenetic relationships and sequence homology (Burgers et al., 2001). In the Euryarchaeota, one of the two main lineages of the domain Archaea, there are two distinct replicative DNA polymerases, family B DNA polymerase (PolB) and family D DNA polymerase (PolD) (Castillo-Lizardo et al., 2014; Greenough et al., 2014; Gueguen et al., 2001). However, species from the Crenarchaeota, the other main archaeal lineage, encode PolB only (Lou et al., 2003; Rogozin et al., 2008). PolB consists of a single subunit containing both 5′→3′ polymerase and 3′→5′ exonuclease activities while PolD is a heterodimer composed of subunits DP1 and DP2, which catalyze proofreading and polymerization, respectively (Gueguen et al., 2001; Shen et al., 2001). PolD is unique to euryarchaea (Tori et al., 2007). Physiological roles of the two distinct DNA polymerases in euryarchaea are still unclear. Henneke et al. showed that the hyperthermophilic euryarchaeon *Pyrococcus abyssi* required both PolB and PolD for the growth and they might function on the leading and the lagging strands, respectively (Henneke et al., 2005). However, Cubonova et al. found that PolB, but not PolD, could be deleted from *Thermococcus kodakarensis*, another hyperthermophilic euryarchaeon, suggesting that PolB is not essential *in vivo* (Cubonova et al., 2013).

A DNA polymerase may be able to catalyze strand displacement, which plays a key role in Okazaki fragment maturation in both eukaryotes and archaea (Beattie and

*Corresponding author (email: huangl@sun.im.ac.cn)

Bell, 2012; Gloor et al., 2012; Sun and Huang, 2013). During the replication of the lagging strand, a polymerizing DNA polymerase displaces the 5′-RNA stretch into a flap structure. Then the flap is cleaved by nuclease so that the RNA primer is removed from the newly synthesized strand (Zheng and Shen, 2011). Eukaryotic DNA polymerase δ (Polδ) is able to displace a 72-nt DNA strand (Maga et al., 2001). ϕ29 DNA polymerase is capable of displacing a strand as long as ~70 kb (Blanco et al., 1989). DNA polymerase B1 from the hyperthermophilic crenarchaeon *Sulfolobus solfataricus* (SsoPolB1) is able to synthesize strand displacement products of ~3,000 nt (Sun and Huang, 2013). Both PolB and PolD from *P. abyssi* possess strand displacement activity, and are able to displace a 30-nt DNA strand in the presence of proliferating cell nuclear antigen (PCNA) (Henneke et al., 2005).

Genomic DNA is organized into chromatin by a group of small, basic and abundant proteins. In crenarchaea, Cren7 serves as the universal chromatin protein (Guo et al., 2007), with additional chromatin proteins existing in some genera (e.g., Sul7d in *Sulfolobus* (Mai et al., 1998) and CC1 in *Aeropyrum* (Luo et al., 2007)). These proteins exist as monomers both in solution and upon binding to DNA (Guo et al., 2007; Luo et al., 2007; Mai et al., 1998). Unlike crenarchaea, euryarchaea possess homologs of eukaryotic histones. In contrast to the octamer of eukaryotic histones, archaeal histones exist as homodimers or heterodimers in solution, and polymerize further to form tetramers in the presence of DNA (Sandman and Reeve, 2005).

Chromatin proteins have recently been shown to modulate strand displacement by DNA polymerase. Smith et al. revealed the function of nucleosomes in Okazaki fragment termination in *Saccharomyces cerevisiae*, and showed that histones served to avoid excess strand displacement by Polδ in Okazaki fragment maturation (Smith and Whitehouse, 2012). More recently, both Sul7d and Cren7 from the crenarchaeon *S. solfataricus* were found to inhibit displacement of a DNA, but not an RNA, strand from template DNA by SsoPolB1 (Sun and Huang, 2013). Since euryarchaea differ distinctively from crenarchaea in chromatin proteins and replicative DNA polymerases, it is of interest to compare the two archaeal lineages in strand displacement by DNA polymerases and its modulation by chromatin proteins. In addition, understanding of the differences between PolD and PolB in their strand displacement ability and their response to the presence of the chromatin protein will shed light on the cooperation of the two polymerases in the process of DNA replication. In this study, we determined the strand displacement activities of both PolB and PolD from *P. furiosus* as well as the role of histone HPfA1 in modulating these activities. Our results show that, although crenarchaea and euryarchaea differ in chromosomal packaging, they share similar mechanisms in modulating strand displacement by DNA polymerases during lagging strand DNA synthesis.

RESULTS

Both PolB and PolD are capable of strand displacement

To test the strand displacement activity of PolB and PolD, we prepared a 72-nt minicircular ssDNA molecule annealed to a 36-nt 5′-radiolabled oligonucleotide primer (P36/C72). A DNA polymerase would extend the radiolabeled primer on this primed minicircle. Once the progressing DNA polymerase encounters the 5′-end of the primer, it would stop if it lacks the strand displacement activity, generating products no longer than 72 nt. On the other hand, the polymerase would continue polymerization, displacing the preexisting labeled strand into a flap and producing fragments longer than 72 nt, if the enzyme has the strand displacement activity (Figure 1). As shown in Figure 2, both PolB and PolD were able to synthesize products of >72 nt, even when the enzyme was added at 5 nmol L^{-1}. Polymerization products became increasingly longer as the polymerase concentration increased to 50 nmol L^{-1}, suggesting a dose dependence of the efficiency of strand displacement by both DNA polymerases. Intriguingly, when the level of polymerase was raised to 100–200 nmol L^{-1}, PolB synthesized still longer products but, at the same time, produced a slightly increased amount of fragments shorter than 36 nt, presumably products of the exonuclease activity of the polymerase. By comparison, the strand displacement products of PolD decreased significantly, while products shorter than 36 nt were apparently extended by the enzyme, when the protein concentration was similarly increased. It appears that PolB and PolD differ in the ability to coordinate their polymerase and exonuclease activities.

Based on these results, PolB and PolD were used at 100 and 50 nmol L^{-1}, respectively, in the following assays to maintain the maximum strand displacement activity and the minimum exonuclease activity of the polymerase.

PolB-mediated strand displacement is modulated by HPfA1

We showed previously that chromatin proteins Sso7d and Cren7 were able to modulate strand displacement by PolB1 from *S. solfataricus* (Sun and Huang, 2013). The euryarchaeon *P. furiosus* lacks Sso7d or Cren7 homologs, but encodes homologs of eukaryotic histones. To learn how an archaeal histone may affect strand displacement by DNA polymerase from a euryarchaeon, we determined strand displacement by PolB or PolD in the presence of HPfA1, a *P. furiosu*s histone.

When the assay was performed under the same conditions as those described for Figure 2, not only strand displacement but also gap-filling by PolB were inhibited by HPfA1 even at a very low concentration (i.e., 0.1 μmol L^{-1}). The majority of the products were between 46 and 72 nt in

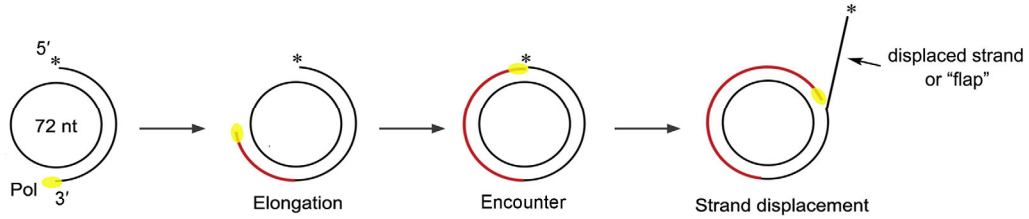

Figure 1 Schematic representation of strand displacement by a DNA polymerase on a 72-nt minicircular primed template. DNA polymerase is shown in yellow, and newly synthesized DNA strand is in red. The radioactive label at the 5′ end of the primer is indicated by an asterisk.

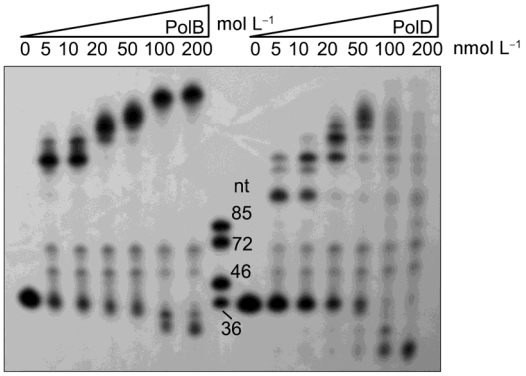

Figure 2 Strand displacement by PolB and PolD on the circular template P36/C72. An indicated amount of PolB or PolD was incubated for 15 min at 70°C with P36/C72 (2 nmol L^{-1}). Samples were subjected to electrophoresis in an 8% polyacrylamide gel containing 7 mol L^{-1} urea in 1×TBE. M, size markers.

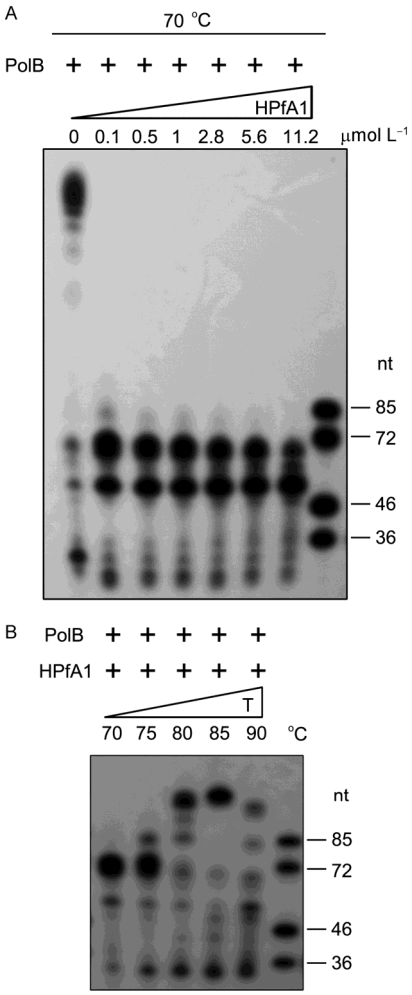

Figure 3 Temperature dependence of the effect of HPfA1 on strand displacement by PolB. A, PolB (100 nmol L^{-1}) was incubated for 15 min at 70°C with P36/C72 (2 nmol L^{-1}) in the presence of HPfA1 at an indicated concentration. B, PolB (100 nmol L^{-1}) was incubated for 15 min at an indicated temperature with P36/C72 (2 nmol L^{-1}) in the presence of 0.1 μmol L^{-1} HPfA1. Samples were treated with proteinase K and extracted with phenol/chloroform. Reaction products were subjected to electrophoresis in an 8% polyacrylamide gel containing 7 mol L^{-1} urea in 1×TBE.

length, and products longer than 72 nt were hardly detectable (Figure 3A). Apparently, primer extension by PolB was blocked before the polymerase could reach the 5′-end of the primer. It was speculated that the observed inhibition might have resulted from the formation of secondary structures by the histone in the single-stranded DNA region of the template. This would interfere with the analysis of the effect of HPfA1 on strand displacement by PolB. To eliminate the interference, we tried to determine the optimal temperature for the assay. Since *P. furiosus* lives optimally at ~100°C (Fiala and Stetter, 1986), a much higher temperature than that used in the above assay, we tested the effect of higher temperatures on strand displacement by PolB in the presence of 0.1 μmol L^{-1} HPfA1. As shown in Figure 3B, little strand displacement was observed at 70°C–75°C. However, when the temperature was increased to 80°C–90°C, strand displacement became significant. We also repeated the assay shown in Figure 3A at 80°C. We found that the sizes of strand displacement products became shorter as more HPfA1 was titrated into the reactions, but gap-filling synthesis by the polymerase was nearly unaffected (Figure 4B). Analysis by sequencing gel showed that PolB was able to synthesize products of the same size on P36/C72 as those on the linear primer-template P36/L72 in the presence of a saturating amount (11.2 μmol L^{-1}) of HPfA1 (Figure 4C), demonstrating that primer extension by PolB was blocked when the enzyme encountered the 5′-end of the primer. In other words, strand displacement by PolB was abolished when the template was maximally bound by HPfA1.

During Okazaki fragment maturation, downstream 5′-RNA primers must be displaced by a DNA polymerase

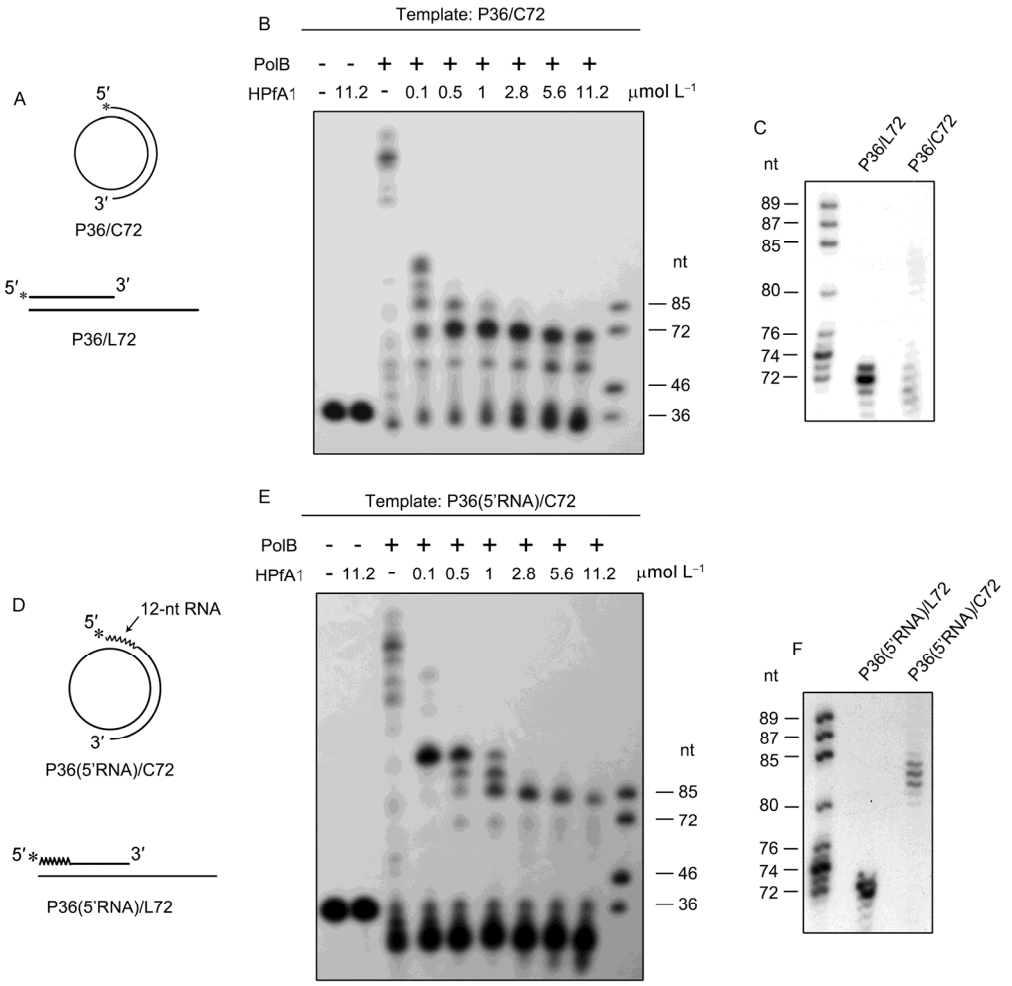

Figure 4 Modulation of PolB-mediated strand displacement on DNA- and RNA-primed templates by HPfA1. A, Schematic representation of DNA-primed templates used in Figure 4B and C. B, PolB (100 nmol L^{-1}) was incubated for 15 min at 80°C with P36/C72 (2 nmol L^{-1}) in the presence of indicated amounts of HPfA1. Samples were treated with proteinase K and extracted with phenol/chloroform. Reaction products were subjected to electrophoresis in 8% polyacrylamide gel containing 7 mol L^{-1} urea in 1×TBE. C, PolB (100 nmol L^{-1}) was incubated for 15 min at 80°C with P36/C72 or P36/L72 (2 nmol L^{-1}) in the presence of 11.2 μmol L^{-1} HPfA1. After incubation with proteinase K and phenol/chloroform extraction, products were concentrated by ethanol precipitation. The sample was dissolved in 5 μL of loading buffer and loaded onto an 8% sequencing gel in 1×TBE. D, Schematic representation of RNA-primed templates used in Figure 4E and F. E and F, Reactions were assembled and processed as described in Figure 4B and C except that RNA-primed templates, instead of DNA-primed templates, were used.

so that they can be removed by a nuclease (Zheng and Shen, 2011). The above experiments showed that HPfA1 abolished DNA strand displacement by PolB. To investigate if HPfA1 also inhibits the displacement of a downstream 5′-RNA primer by PolB, we used in the assays the primer-template P36 (5′RNA)/C72, which is identical to P36/C72 except for the replacement of the 5′-end 12-nt sequence of the primer with an RNA stretch (Figure 4D). As shown in Figure 4E, strand displacement by PolB on P36 (5′RNA)/C72 was also inhibited by HPfA1, but the pattern of inhibition was different from that observed for that on P36/C72. The lengths of the products on P36 (5′RNA)/ C72 were close to 85 nt rather than 72 nt. Sequencing gel data revealed that these products ranged in size from 82 to 84 nt (Figure 4F), suggesting the displacement of a stretch of 10–12 nt, which corresponded to the RNA at the 5′-end of the primer. Therefore, we conclude that HPfA1 inhibits only DNA, and not RNA, displacement by PolB.

HPfA1 modulates strand displacement by PolD

We next studied the effect of HPfA1 on the strand displacement activity of PolD. As found with PolB, gap-filling synthesis by PolD on P36/C72 was inhibited by HPfA1 at 70°C (data not shown). In a control experiment, we found that PolD was active at 75°C, but the activity of the enzyme decreased significantly at 80°C–85°C (Figure 5A). When the assay was conducted in the presence of HPfA1 at 75°C or 80°C, primer extension was still blocked before PolD reached the 5′-end of the downstream primer (Figure 5B). To minimize the potential influence of secondary structures

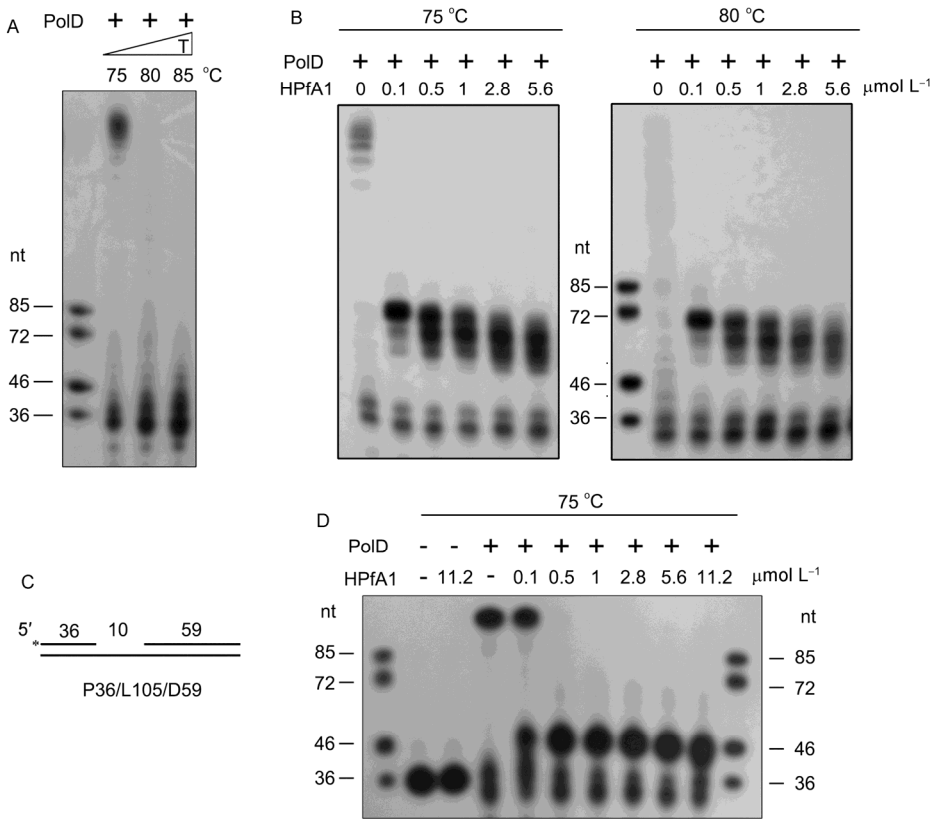

Figure 5 Modulation of PolD-mediated strand displacement by HPfA1. A, Effect of temperature on the strand displacement activity of PolD. PolD (50 nmol L^{-1}) was incubated for 15 min at an indicated temperature with P36/C72 (2 nmol L^{-1}). B, Effect of HPfA1 on PolD-mediated strand displacement on P36/C72 at 75 and 80°C. PolD (50 nmol L^{-1}) was incubated for 15 min at 75 or 80°C with P36/C72 (2 nmol L^{-1}) in the presence of indicated amounts of HPfA1. C, Schematic representation of a primed template used in Figure 5D. D, Effect of HPfA1 on PolD-mediated strand displacement on P36/L105/D59. PolD (50 nmol L^{-1}) was incubated for 15 min at 75°C with P36/L105/D59 (2 nmol L^{-1}) in the presence of indicated amounts of HPfA1.

that might be stabilized by HPfA1 in a circular template on polymerization by PolD, we chose to test the linear primer-template P36/L105/D59, in which a 105-nt linear template (L105) was annealed to a 5′-end primer (P36) and a 59-nt downstream ssDNA (D59) (Figure 5C). Products exceeding 46 nt in length would indicate strand displacement by PolD. As shown in Figure 5D, most of the extension products were longer than or equal to 46 nt, indicating that gap-filling by PolD was no longer efficiently blocked by HPfA1 on this primer-template. The sizes of the products decreased as the HPfA1 concentration increased and were limited to 46 nt in the presence of a saturating amount (11.2 μmol L^{-1}) of HPfA1, indicating that HPfA1 inhibited strand displacement by PolD.

HPfA1 shows greater affinity for a dsDNA fragment than for an RNA∶DNA hybrid

We previously suggested that the ability of both Cren7 and Sul7d, chromatin proteins from *S. solfataricus*, to inhibit DNA, but not RNA, strand displacement by DNA polymerase might have resulted from the higher affinity of the two proteins for dsDNA than for RNA:DNA hybrid (Sun and Huang, 2013). In this study, we compared the binding affinity of HPfA1 for a 30-bp dsDNA and that for a 30-bp RNA:DNA hybrid, whose DNA strand was identical to one of the two strands in the dsDNA. Like the *S. solfataricus* chromatin proteins, HPfA1 bound more strongly to the dsDNA than to the RNA:DNA hybrid (Figure 6). This is consistent with the notion that the nucleic acid binding preference of HPfA1 is responsible for the different effects of the protein on DNA and RNA displacement by the two *P. furiosus* DNA polymerases.

DISCUSSION

Crenarchaeota and Euryarchaeota represent two major lineages in the domain Archaea. Despite their overall similarity in genetic mechanisms, they differ significantly in the use of DNA polymerases in DNA replication. While PolB, a family B DNA polymerase, is employed by both crenarchaea and euryarchaea, PolD, a unique family D DNA polymerase functions in DNA replication only in the latter (Shen et al., 2001). The use of two distinct DNA polymerases for DNA replication in euryarchaea is reminiscent of that in eukaryotes, in which Polε and Polδ catalyze DNA synthesis on the leading and the lagging strands, respectively (Nick Mc-

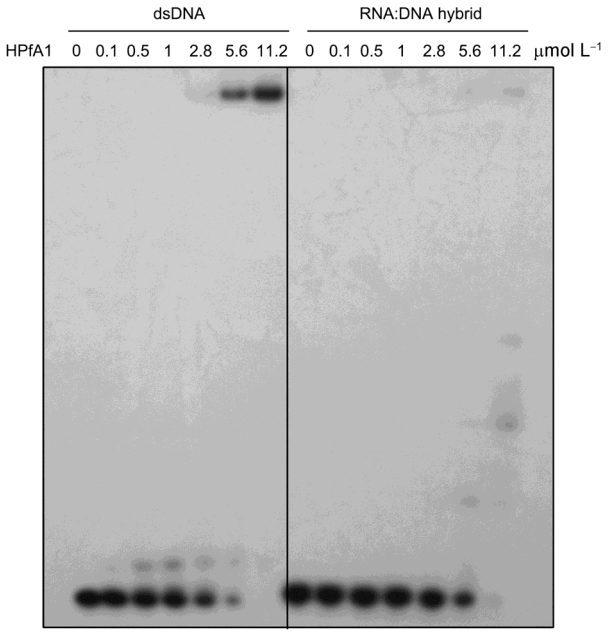

Figure 6 Binding of HPfA1 to a dsDNA fragment and a RNA:DNA hybrid. An indicated amount of HPfA1 was incubated for 10 min at 23°C with a 30-bp dsDNA or RNA:DNA hybrid (2 nmol L^{-1}). Samples were subjected to electrophoresis in a native 5% polyacrylamide gel in 0.1×TBE.

Elhinny et al., 2008; Pursell et al., 2007). A possibility arises that PolB and PolD also have different roles in DNA replication in euryarchaea. Since a lagging strand DNA polymerase is capable of efficient strand displacement synthesis, which is essential for Okazaki fragment maturation (Maga et al., 2001), the difference between PolB and PolD in strand displacement activity would provide a clue as to which of the two polymerases might be involved in lagging strand synthesis. However, in this study, we found that both PolB and PolD were able to catalyze strand displacement. Therefore, it is impossible to assign different roles to the two DNA polymerases based on the results of the strand displacement assays. Our data contrast with a previous study in which PolD, but not PolB, was shown to have a strand displacement activity, an observation which prompted the suggestion that PolB and PolD contributed to leading and lagging strand replication, respectively, in *Pyrococcus abyssi*, a hyperthermophilic euryarchaeon closely related to *P. furiosus* (Henneke et al., 2005). The discrepancy may result from the difference in the experimental conditions employed in the two studies. The assays were performed at 50°C in the previous study and 70°C in the present study. Thus, it is possible that PolB activity was substantially underestimated at 50°C.

Euryarchaea also appear to differ from crenarchaea in genome packaging. While the former encode archaeal histones, the latter employ unique chromatin proteins, such as Cren7 and Sul7d (Zhang et al., 2012). Despite their differences in sequence and structure, these archaeal chromatin proteins behaved in a similar fashion in modulating strand displacement. As observed previously with Cren7 and Sul7d (Sun and Huang, 2013), HPfA1, a histone homolog from *P. furiosus*, was able to inhibit DNA strand displacement by either PolB or PolD. Notably, however, although PolB and PolD were similarly active in strand displacement, they differed in response to the inhibition by HPfA1. For example, gap-filling synthesis on P36/C72 by both PolB and PolD was inhibited by HPfA1 at a sub-saturating level (0.1 μmol L^{-1}) at 70°C. When the temperature was raised to >80°C, gap-filling synthesis by PolB occurred efficiently but the synthesis by PolD remained inhibited. Presumably, during primer extension, PolB is more proficient than PolD in overcoming the hindrance of the secondary or higher-order structures generated on the template as the result of binding by HPfA1. Given that secondary or higher-order structures are more likely formed on the lagging strand than on the leading strand, PolB appears to be a better candidate for a role in lagging strand synthesis than PolD.

Although HPfA1 inhibited DNA strand displacement, it did not affect RNA strand displacement by PolB, as observed for the modulating role of Cren7 and Sul7d in the strand displacement activity of PolB1 from *S. solfataricus* (Sun and Huang, 2013). This difference is consistent with the different affinities of HPfA1 for dsDNA and RNA:DNA hybrid, as found with the chromatin proteins from *S. solfataricus* (Sun and Huang, 2013). Taken together, our results suggest that crenarchaea and euryarchaea may differ in chromosomal packaging, but they share similar mechanisms in modulating strand displacement by DNA polymerases during lagging strand DNA synthesis.

MATERIALS AND METHODS

Expression plasmids

The gene encoding HPfA1 (open reading frame PF1831) was amplified by PCR from the genomic DNA of *P. furiosus* DSM 3638 using Taq DNA polymerase (TaKaRa, Dalian) and the following pair of primers: 5′-CCG**CATATG**-GGAGAATTGCCAATTGCCCCAGT/5′-GCC**GGATCC**T CAGCTCTTAATTGCGAGCTTAATGTCTTCAACCT (*Nde*I and *Bam*HI sites are in bold face). The PCR product was ligated into pGEM-T (Promega, USA). The recombinant plasmid was cleaved with *Nde*I and *Bam*HI. The fragment containing the HPfA1 gene was inserted into the same sites on the expression vector pET-30a(+) (Novagen, USA), yielding expression plasmid pET30a-HPfA1. Expression plasmids for PolB, DP1 and DP2 (pET21d-PolB, pET28a-DP1 and pET21a-DP2, respectively) were generous gifts from Professor Yoshizumi Ishino (Kyushu University, Japan).

Proteins

Recombinant HPfA1 was prepared as described previously for HPhA (Weng et al., 2004). Recombinant PolB and PolD

were prepared as described previously (Tori et al., 2007) with modifications. For PolB, *E. coli* Rosseta (DE3) cells (TransGen Biotech, Beijing) carrying pET21d-PolB were grown at 30°C in Luria-Bertani (LB) medium containing 100 μg mL^{-1} ampicillin and 35 μg mL^{-1} chloramphenicol. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mmol L^{-1} when the cell density of the culture reached an A_{600} of 0.5. Incubation was continued for overnight at 24°C. Cells were harvested, resuspended in buffer A (50 mmol L^{-1} Tris-HCl, pH 8.0, 0.1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 0.5 mmol L^{-1} dithiothreitol (DTT), 10% glycerol), and disrupted by sonication. The cell extract was heated for 20 min at 80°C. The heat-treated sample was centrifuged, and the supernatant was loaded onto a HiTrap S column (GE Healthcare) equilibrated in buffer A. The column was developed with a linear gradient of 0 to 0.35 mol L^{-1} NaCl. Fractions containing PolB were pooled and dialyzed against buffer A. The dialysate was subsequently subjected to chromatography on a HiTrap Q column (GE Healthcare) equilibrated in buffer A. Proteins were eluted with a gradient of 0–0.4 mol L^{-1} NaCl. Fractions containing PolB were pooled, dialyzed against buffer A and stored at −80°C. For PolD, pET28a-DP1 and pET21a-DP2 were co-transformed into *E. coli* Rosseta (DE3) cells. The transformant was grown at 37°C to an A_{600} of 0.6 in LB medium containing 100 μg mL^{-1} ampicillin, 50 μg mL^{-1} kanamycin and 35 μg mL^{-1} chloramphenicol. IPTG (1 mmol L^{-1}) was added and incubation was continued for an additional 7 h at 37°C or for overnight at 24°C. Cells were harvested, resuspended in buffer B (50 mmol L^{-1} Tris-HCl, pH 8.0, 0.5 mol L^{-1} NaCl, 10% glycerol) and disrupted by sonication. After a 20-min incubation at 80°C, the sample was centrifuged. The supernatant was subjected to a HisTrap column equilibrated in buffer B. Proteins were eluted with a linear gradient of 0–500 mmol L^{-1} imidazole. Fractions containing PolD (DP1 + DP2) were pooled and dialyzed against buffer C (50 mmol L^{-1} Tris-HCl, pH 8.0, 0.1 mol L^{-1} NaCl, 0.1 mmol L^{-1} EDTA, 0.5 mmol L^{-1} DTT, 10% glycerol). Further purification was carried out by chromatography on a HiTrap Q colunm, followed by an additional purification step using a HiTrap Heparin column. Both columns were developed with a gradient of 0–1 mol L^{-1} NaCl. Fractions containing PolD were pooled, dialyzed against buffer C and stored at −80°C.

Polymerase templates

The sequences of oligonucleotides used in this study are shown in Table 1. Oligodeoxyribonucleotides were synthesized at Sango BioTech (Shanghai). RNA-containing oligonucleotides were synthesized at TaKaRa (Dalian). A 72-nt minicircular ssDNA molecule was constructed as described previously (Sun and Huang, 2013). To prepare a primer-template, 5′-^{32}P-labeled P36 or P36 (5′-RNA) was mixed with L72 or C72 at a molar ratio of 1∶1.5 in 20 mmol L^{-1} Tris-HCl, pH 8.0, and 100 mmol L^{-1} NaCl. To prepare a linear primer-template containing a downstream 3′-duplex region (P36/L105/D59), 5′-radiolabled P36, L105 and D59 were mixed at a molar ratio of 1∶1.5∶2. The above mixtures were boiled for 3 min and left to cool down slowly to room temperature.

DNA polymerase assays

The standard reaction (20 μL) contained 2 nmol L^{-1} primer-template, 20 mmol L^{-1} Tris-HCl, pH 8.8, 10 mmol L^{-1} KCl, 10 mmol L^{-1} $(NH_4)_2SO_4$, 2 mmol L^{-1} $MgSO_4$, 0.1% Triton X-100, 0.1 mg mL^{-1} BSA and 1 mmol L^{-1} dNTPs (Roche). DNA polymerase (PolB or PolD) were added as indicated in the figure legend. HPfA1 was included in the reaction when specified. The mixtures were incubated for 15 min at an indicated temperature. The reaction containing no HPfA1 was quenched by an equal volume of 2×loading buffer (95% deionized formamide, 100 mmol L^{-1} EDTA, 0.025% bromphenol blue and 0.025% xylene cyanol FF). When the reaction contained HPfA1, a solution (4 μL) containing 3% SDS, 150 mmol L^{-1} EDTA and 15 mg mL^{-1} proteinase K (Promega) was added to the sample. The mixture was incubated for 45 min at 50°C to digest the chromatin protein, and extracted with phenol/chloroform. The sample was then mixed with the loading buffer. After boiling

Table 1 Oligonucleotides used in this study[a)]

Name	Sequence (5′→3′)
P36	TGCATCCTTCAATGTGCTGGGATCCTACAACCAAGA
P36 (5′RNA)	*UGCAUCCUUCAATGTGCTGGGATCCTACAACCAAGA
L72	CTTCTAGTTGTGAATTCGGCACTGGCCGTCGTATGCTCTTGGTTGTAGGATCCCAGCACATTGAAGGATGCA
L105	ATTCGGCACTGGCCGTCGTATGCTCTTGGTTGTAGGATCCCAGCACATTGAAGGATGCACTTCTAGTTGTGAACCGCGCCA GCGACATCATGCAAGGTCTCCAAG
D59	TGCATCCTTCAATGTGCTGGGATCCTACAACCAAGAGCATACGACGGCCAGTGCCGAAT
D30	TGCTTCCTTCAATGTGCTGGGATCCTACAA
R30	UGCUUCCUUCAAUGUGCUGGGAUCCUACAA
Com30	TTGTAGGATCCCAGCACATTGAAGGAAGCA

a) *, ribonucleotides are underlined.

for 3 min and subsequent cooling on ice, the sample was subjected to electrophoresis in 8% denaturing polyacrylamide gel containing 7 mol L^{-1} urea in 1×Tris borate-EDTA (TBE). For sequencing gel electrophoresis, the sample was precipitated with ethanol, dissolved in the loading buffer and resolved by 8% denaturing PAGE containing 7 mol L^{-1} urea in 1×TBE. The gel was dried and exposed to X-ray film.

Electrophoretic mobility shift assay (EMSA)

A ^{32}P-labeled oligonucleotide (D30 or R30) was annealed to its complementary ssDNA (Com30) at a molar ratio of 1∶1.5, yielding a 30-bp dsDNA or 30-bp RNA:DNA hybrid fragment. Indicated amounts of HPfA1 were incubated with a radiolabeled nucleic acid fragment (2 nmol L^{-1}) for 10 min at room temperature in 20 mmol L^{-1} Tris-HCl, pH 8.8, 10 mmol L^{-1} KCl, 10 mmol L^{-1} $(NH_4)_2SO_4$, 2 mmol L^{-1} $MgSO_4$, 0.1% Triton X-100, 0.1 mg mL^{-1} BSA and 5% glycerol (v/v). The protein-nucleic acid complexes were electrophoresed in 5% polyacrylamide gel in 0.1×TBE. Following electrophoresis, the gel was dried and exposed to X-ray film.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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