ABBS

Original Article

Translesion synthesis of apurinic/apyrimidic site analogues by Y-family DNA polymerase Dbh from *Sulfolobus acidocaldarius*

Weiwei Wang[1,2,†], Huan Zhou[1,2,†], Li Peng[3,†], Feng Yu[1,2], Qin Xu[1,2], Qisheng Wang[1,2,*], Jianhua He[1,2,*], and Xipeng Liu[3,*]

[1]Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China, [2]University of Chinese Academy of Sciences, Beijing 100049, China, and [3]State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[†]These authors contributed equally to this work.
*Correspondence address. Tel: +86-21-34204378; E-mail: xpliu@sjtu.edu.cn (X.L.) / Tel: +86-21-33933192; E-mail: wangqisheng@zjlab.org.cn (Q.W.) / Tel: +86-21-33933186; E-mail: hejh@sinap.ac.cn (J.H.)

Abstract

Apurinic/apyrimidic (AP) sites are severe DNA damages and strongly block DNA extension by major DNA polymerases. Y-family DNA polymerases possess a strong ability to bypass AP sites and continue the DNA synthesis reaction, which is called translesion synthesis (TLS) activity. To investigate the effect of the molecular structure of the AP site on the TLS efficiency of Dbh, a Y-family DNA polymerase from *Sulfolobus acidocaldarius*, a series of different AP site analogues (various spacers) are used to characterize the bypass efficiency. We find that not only the molecular structure and atomic composition but also the number and position of AP site analogues determine the TLS efficiency of Dbh. Increasing the spacer length decreases TLS activity. The TLS efficiency also decreases when more than one spacer exists on the DNA template. The position of the AP site analogues is also an important factor for TLS. When the spacer is opposite to the first incorporated dNTPs, the TLS efficiency is the lowest, suggesting that AP sites are largely harmful for the formation of hydrogen bonds. These results deepen our understanding of the TLS activity of Y-family DNA polymerases and provide a biochemical basis for elucidating the TLS mechanism in *Sulfolobus acidocaldarius* cells.

Key words translesion synthesis, *Sulfolobus acidocaldarius*, Y-family DNA polymerase, Dbh, AP site analogues

Introduction

Genomic DNA replication is an extraordinarily complex and highly ordered enzymatic reaction process that is accomplished by dozens of proteins. DNA polymerase plays a core role during the replication of genomic DNA. In addition to chromosome replication, DNA polymerase also participates in DNA repair. According to sequence similarity, DNA polymerases are divided into six families: A, B, C, D, X and Y [1]. Each DNA polymerase family has a different structure and function [2–4] but they all incorporate dNMPs into the 3'-OH group of a primer [3]. Bacteria, archaea, and eukaryotes use different DNA polymerases to replicate chromosomal DNA. Bacteria and eukaryotes use family C and family B DNA polymerases to replicate the chromosome, respectively [5]. In archaea, DNA poly-

merases of family D and family B (not all members) are responsible for genome replication [6]. DNA polymerases of other families are mainly involved in repairing various types of DNA damage.

Various DNA repair pathways, including base excision repair, nucleotide excision repair, and homologous recombination, can repair most types of DNA damage [7,8]. However, DNA damage can also escape the repair system, so tolerance of such damage is important for cell survival [9,10]. DNA damage tolerance is mainly achieved through two approaches: error-free and error-prone pathways [10]. In the error-free pathway, DNA polymerase usually synthesizes a complementary strand with some error bases, which are replaced with the correct bases through subsequent homologous recombination repair [11]. However, the error-free pathway can

introduce some other forms of genetic instability, such as hetero-topic recombination between scattered repetitive sequences [12]. In contrast, error-prone DNA damage tolerance is realized by by-passing the damage site and incorporating error bases, leading to translesion synthesis (TLS). A specific kind of DNA polymerase can extend the daughter DNA strand to bypass the damage in the tem-plate strand in the error-prone pathway [13]. These DNA poly-merases are called TLS polymerases and generally belong to the Y family [11].

TLS polymerases from the Y family have a more flexible catalytic centre and lower fidelity, allowing them to insert imperfectly mat-ched nucleotides opposite the DNA damage site while also in-troducing more errors during replication of normal DNA templates [14–16]. The low fidelity increases the frequency of base muta-genesis. However, base mutagenesis can be minimized by other characteristics of TLS polymerases, including their low processive synthesis ability and their tendency to insert the correctly paired nucleotide with a specific kind of DNA damage [17]. Extension of severely damaged DNA by replicative DNA polymerase is blocked, resulting in replicative fork collapse [18]. When the replicative fork encounters severe DNA damage, the TLS polymerase is recruited as a substitute for the replicative DNA polymerase and performs TLS until bypassing the damage [18,19]. Then, the replicative fork is immediately reconstituted for high-fidelity DNA replication by switching to replicative DNA polymerase. These Y-family poly-merases have low sequence similarity, usually exhibiting less than 30% sequence homology between different subfamilies or less than 40% sequence homology in the same subfamily, so each poly-merase is generally endowed with different characteristics [20]. Although Y-family DNA polymerases have different preferences for the incorporated nucleotides [14,21], they generally can incorporate nucleotides accurately while bypassing the damage [22,23].

Sulfolobus acidocaldarius, isolated from the acidic hot springs of Yellowstone National Park in the USA, belongs to the Crenarchaeota phylum [24–26]. Because S. acidocaldarius lives in hot and acidic environment, it encounters a large amount of DNA damage [24,25], which imposes a considerable burden on the maintenance of gen-ome integrity [27,28]. The Dbh protein, a DNA polymerase IV from S. acidocaldarius, has been investigated intensively in vitro as a biochemical and structural model of translesion synthesis [29] and can bypass some DNA damages, such as abasic sites, often gen-erating single-base deletions [30].

To completely understand the TLS of apurinic/apyrimidic (AP) site damage, we characterized the TLS of various AP site analogues by the archaeal DNA polymerase Dbh. The results show that the spacer molecular structure is a key element determining the TLS efficiency of Dbh. TLS is also affected by the spacer length, position, and number. Our results provide a biochemical basis for under-standing TLS function in S. acidocaldarius cells.

Materials and Methods
Materials
The S. acidocaldarius strain used in this study was provided by Professor Sonja-Verena Albers from the Laboratory of Molecular Biology of Archaea, Institute of Biology, University of Freiburg (Freiburg, Germany). Its genomic DNA was extracted by using phenol-chloroform. The expression vector pDEST17 was used to express recombinant Dbh. The Escherichia coli strains DH5α and Rosetta2(DE3)pLysS were used for gene cloning and recombinant

protein expression, respectively. PrimeSTAR Max DNA polymerase was purchased from TaKaRa (Shiga, Japan). Nickel–nitrilotriacetic acid resin was purchased from Bio-Rad (Hercules, USA). The DNA oligonucleotide substrates used for biochemical characterization of Dbh activity are shown in Supplementary Table S1 and were syn-thesized by Biosune (Shanghai, China). All other chemicals and reagents were of analytical grade.

Preparation of recombinant Dbh
The S. acidocaldarius dbh (ORF Saci0554) gene was amplified from genomic DNA by PCR using a forward primer (5′-GGCTCCCATA TGTCCAAACAAGCAACACT-3′) and a reverse primer (5′-GGATC CGAATTCTCATTTCTTACTTCCGAAGA-3′) and then inserted into pDEST17 as previously described [31], producing the recombinant expression vector pDEST17-Dbh. DNA sequencing was used to confirm the sequence of the inserted dbh gene in the expression plasmids.

The pDEST17-Dbh expression plasmid was introduced into E. coli Rosetta2(DE3)pLysS to express the recombinant Dbh protein. Iso-propyl-1-thio-β-D-galactopyranoside (0.5 mM, final concentration) was added to the bacterial cultures ($OD_{600} = 0.8$) to induce re-combinant protein expression, and the cells were grown for 20 h with shaking at 20°C. The cultures were harvested and resuspended in ice-cold lysis buffer [20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol, 5 mM imidazole, 1 mM phe-nylmethylsulfonyl fluoride (PMSF) and 10% glycerol] before lysing the cells by sonication. After incubation for 15 min at 75°C, the lysates were clarified by centrifugation at 16,000 g for 30 min at 4°C, and then, the supernatants were purified by immobilized metal affinity chromatography. After the supernatant was loaded onto a column pre-equilibrated with lysis buffer, the resin was washed with 100 column volumes of lysis buffer containing 25 mM imida-zole. Finally, the bound Dbh was eluted from the column using elution buffer (20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5 mM β-mercaptoethanol, 300 mM imidazole and 10% glycerol). The purity of the eluted Dbh was confirmed by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The preparations were further dialyzed against storage buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl and 50% glycerol) and then stored in small ali-quots at –20°C.

Preparation of the substrates for the TLS activity assay
The primer-template DNAs were prepared by annealing 5′-FAM-labelled primer strands to AP site-containing template strands in a molecular ratio of 1:1.5 by boiling for 5 min at 95°C and slowly cooling down to room temperature, and used as substrates for the TLS assay. The oligodeoxyribonucleotide templates contained dif-ferent AP site analogues (alkane chain, polyethylene glycol, dSpa-cer, rSpacer, and Dual SH) and were used to determine the effect of the molecular structural characteristics of AP site analogues on the TLS. The natural AP site deoxyribose phosphate (dRP) was pre-pared by treating the dU-carrying oligodeoxyribonucleotide with uracil-DNA glycosylase (UDG) as previously described [21]. The alkane chains are linear analogues of the natural AP site deoxyr-ibose. Polyethylene glycol is a variant of the alkane chain obtained by introducing an oxygen atom into the carbon chain. dSpacer is a terahydrofuran analogue of the natural AP site. rSpacer is a 2′-OH analogue of the natural AP site. Dual SH is an analogue of the C2 chain with one mercapto group on each carbon atom.

Biochemical characterization of TLS activity

The TLS activity of Dbh was determined as previously described [32] with some modifications. Standard reactions (20 μL) contained 100 nM substrate (calculated as the 5′-FAM-labelled primer) and 50 nM Dbh in buffer containing 20 mM Tris-HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 5 mM $MgCl_2$, 0.1% Triton X-100, 0.1 mg/mL BSA, and 0.2 mM dNTPs. The reaction mixtures were incubated at 45°C for the indicated time and were stopped by addition of an equal volume of loading buffer (50 mM EDTA, 8 M urea, 0.2% SDS, 0.1% bromophenol blue, 0.1% xylene cyan) to the reaction samples [32]. Then, the reaction products were analysed by electrophoresis on 15% denaturing polyacrylamide gels containing 8 M urea. After electrophoresis, the gels were imaged using a fluorescent scanner FL9500 (GE Healthcare, Waukesha, USA). The full-length extended products were quantitated by dividing the substrates.

Results

Alkane chain length affects TLS by Dhb

The natural AP site is unstable at high temperature, so it is usually substituted with several AP site analogues in the activity assay [33]. Alkane chain spacers are composed of different numbers of linear C-C bonds. To investigate the bypass of alkane chains by Dbh, oli-godeoxyribonucleotides internally containing various alkane chain spacers of different lengths (C2, C3, C4, C6, C9, and C12) were used as DNA templates to detect TLS activity. For the normal DNA template (adenine base), Dbh easily extended the 17-nt primer to a 26-nt full-length product (Figure 1, panel 1). For the alkane chain spacers, the percentage of the 25-nt full-length extended product band decreased gradually with increasing alkane chain spacer length (Figure 1, panels 2–7) and sharply decreased when the carbon number of the alkane chain was more than 6, indicating that the length of the alkane chain spacer is the major factor determining the TLS efficiency of Dbh. When the number of carbon atoms in the spacer reached 6, the TLS efficiency of Dbh decreased sharply. The natural AP site dRP was bypassed with a similar efficiency as spacer C3 (Figure 1, panel 8). For DNA templates with the normal base or spacers shorter than four carbon atoms, bands longer than the template were generated, suggesting that Dbh has strong template-independent extension activity (Figure 1, panels 1–4,8). The template-independent extension of a blunt double-stranded DNA was verified. The results confirmed that Dbh has template-independent polymerization activity similar to that of *Taq* DNA polymerase (Supplementary Figure S1). Slightly different from *Taq* DNA polymerase, Dbh prefers dAMP and can incorporate up to two dAMPs. On the other hand, *Taq* DNA polymerase can efficiently incorporate
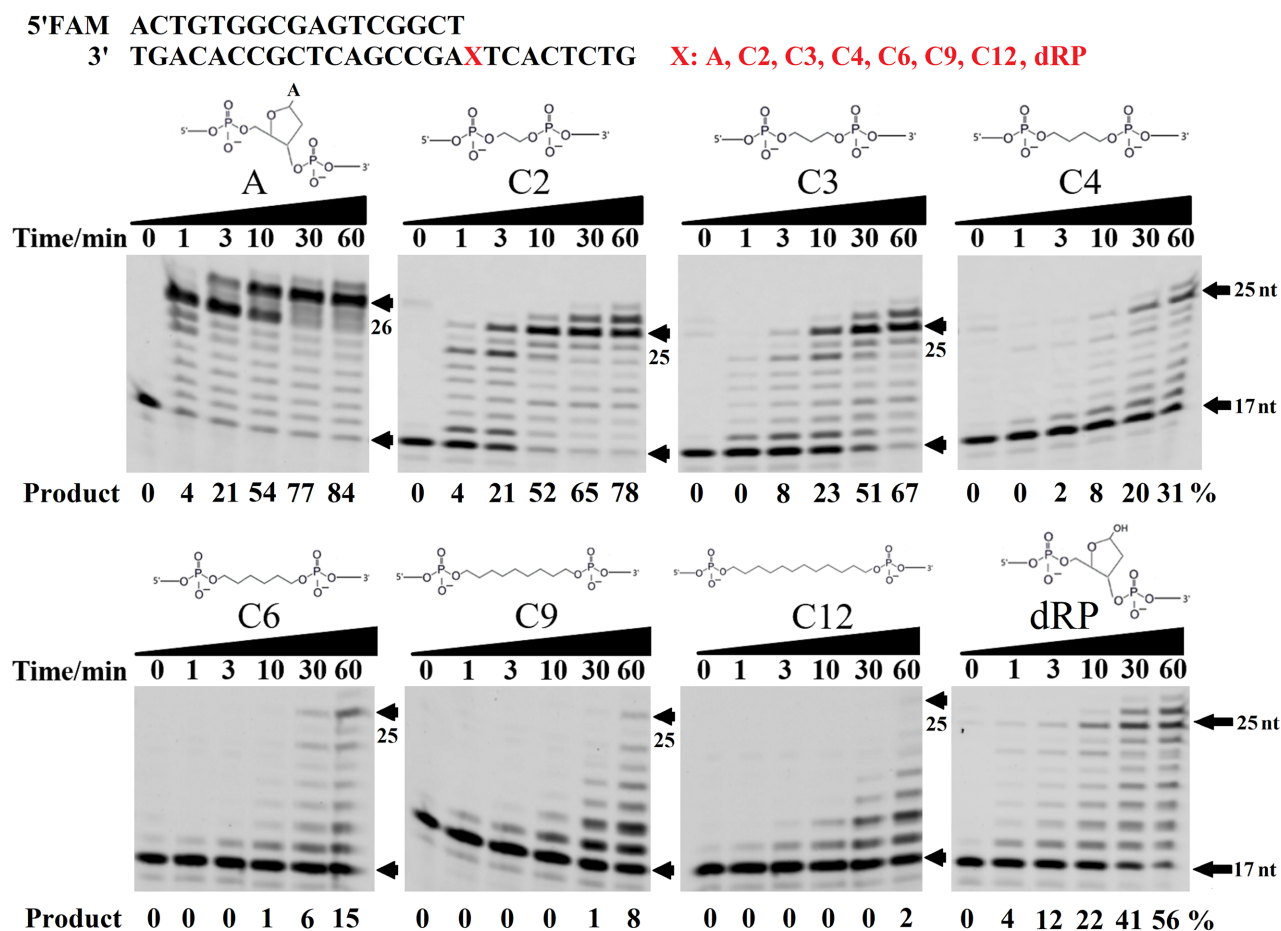
Figure 1. TLS of different alkane chain spacers The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating the normal base A, natural AP site dRP, or the AP site analogues of spacers C2, C3, C4, C6, C9, and C12. The molecular structures of each spacer are shown above each gel diagram. The primers were annealed to each DNA template strand and extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min. The bands of the 17-nt primer and 25-nt (AP sites) or 26-nt (A base) full-length products are indicated by arrows.
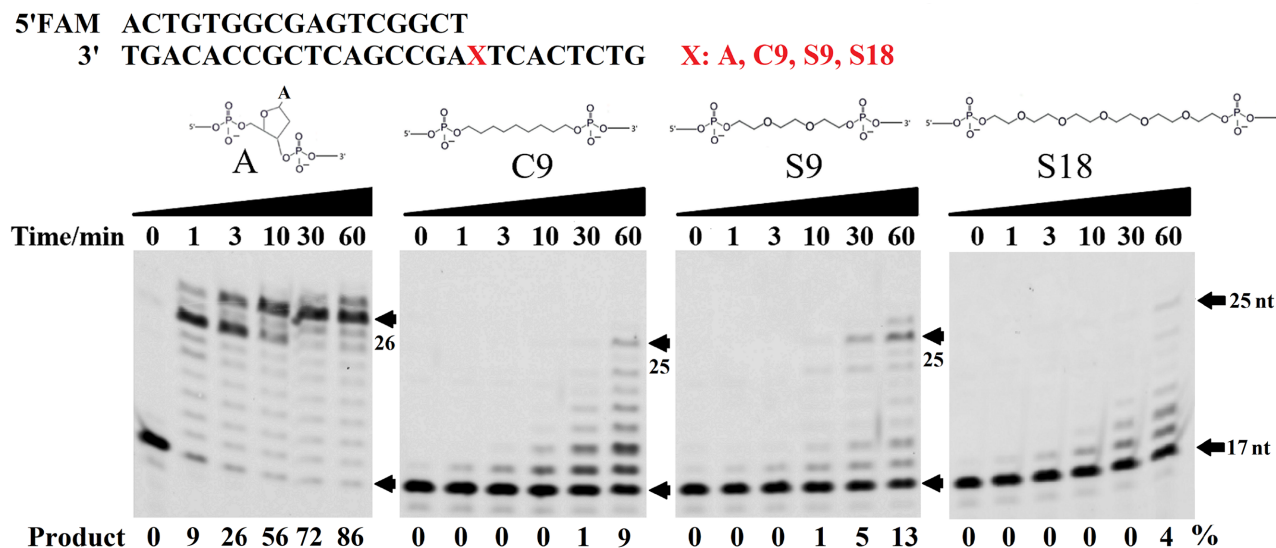
Figure 2. TLS of different polyethylene glycol chain spacers The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating the normal base A or the AP site analogues of spacers C9, S9, and S18. The molecular structures of each spacer are shown above each gel diagram. The primers were annealed to each DNA template strand and extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min.The bands of the 17-nt primer and 25-nt (AP sites) or 26-nt (A base) full-length products are indicated by arrows.

dAMP, dGMP, and dTMP at the 3′-OH of blunt double-stranded DNA.

To further investigate the effect of the number of alkane chain spacers on TLS, five DNA template strands carrying more than one serial spacer C2 or C12 were used to characterize the bypass activity. The results showed that the TLS efficiency of Dbh decreased with increasing numbers of spacer C2 or C12 (Supplementary Figure S2). Considering that the phosphate between two tandem spacers can also function as a spacer, spacers C2 × 2 and C2 × 3 are 7 and 12 atom spacers, respectively. The TLS efficiency of spacer C2 × 3 was slightly higher than that of spacer C12, suggesting that the internal phosphate groups between spacers have promotion effects on the bypass of the whole spaces. In addition, the effect of the combination order of different alkane chain spacers on TLS was characterized. For different spacer combination orders of spacers C3 and C6, the results showed no significant effect on the TLS (Supplementary Figure S3). Similar to the result for serial spacer C2 (Supplementary Figure S2), the TLS efficiency of the combined spacers C3 and C6 was higher than that of the long spacer C12.

Atom composition affects TLS by Dhb

To further investigate the effect of the atomic composition of AP site analogues on TLS, DNA templates with internal spacers of polyethylene glycol and alkane chains were used to compare the bypass activity of Dbh. For the S9 polyethylene glycol and C9 alkane chain spacers, which are of equal length, the TLS of spacer S9 was slightly higher than that of spacer C9 (Figure 2, panels 2,3), suggesting that the oxygen atoms in the polyethylene glycol spacer may facilitate TLS by Dbh. Among the polyethylene glycol spacers, spacer S9 was more easily bypassed than spacer S18 (Figure 2, panels 3,4), indicating that the TLS efficiency decreases with increasing length of polyethylene glycol.

Furthermore, the TLS of DNA template strands carrying ≥3 serial spacers was analyzed. The results showed that the TLS efficiency of multiple serial spacers consisting of alkane chains and polyethylene glycol spacers decreased sharply compared with that of the single

spacer, and only 2–3 nucleotides were added to the 3′-terminus of the primer (Supplementary Figure S4). These results are reasonable because multiple serial spacers are equal or similar to a normal terminus. Then, to test the effect of the number of normal bases between two spacers on TLS, DNA templates with an increasing number of normal bases between the two spacer C6 or S9 were used to analyze the TLS activity. The results showed that for both spacers C6 and S9, the TLS efficiency clearly increased with an increase in the number of normal bases between the two identical spacers (Supplementary Figure S5). Another TLS-related phenomenon was that two bypasses occurred if ≥2 serial bases existed between two identical spacers, and most extension products, indicated by the red arrows in the figure, were blocked at the first spacer (Supplementary Figure S5, panels 2,3,5,6).

The molecular structure affects TLS by Dhb

The linear spacer C3 and the two cyclic spacers dSpacer and rSpacer all have three carbon atoms between the 5′ and 3′ phosphate groups. Therefore, they were used to investigate the effect of cyclic and linear molecular structural features on TLS. The results showed that spacer C3 was bypassed at a little higher efficiency than dSpacer by Dbh (Figure 3, panels 2,3), indicating that the cyclic structure of dSpacer, which is similar to the natural AP site dRP, has no promotion effect on TLS. In addition, Dbh showed a clear preference for spacer C3 and dSpacer, rather than the cyclic deoxyribose-like rSpacer (Figure 3, panels 2–4). Although both rSpacer and dSpacer have cyclic molecular structures, the cyclic ribose structure of rSpacer is different from the cyclic tetrahydrofuran structure of dSpacer, leading to different TLS activities of Dbh (Figure 3).

The dual mercapto groups affect TLS by Dhb

We then compared the effect of the sulphur atomic side chain on TLS by analyzing the bypass efficiencies of spacer C2 and the spacer with two ring-opening free *mercapto* groups (dual-SH). The results showed that the two *mercapto* groups clearly decreased the TLS activity of Dbh (Figure 4), implying that the sulphur atom and/or

5'FAM ACTGTGGCGAGTCGGCT
3' TGACACCGCTCAGCCGA**X**TCACTCTG   **X: A, C3, dSpacer, rSpacer**
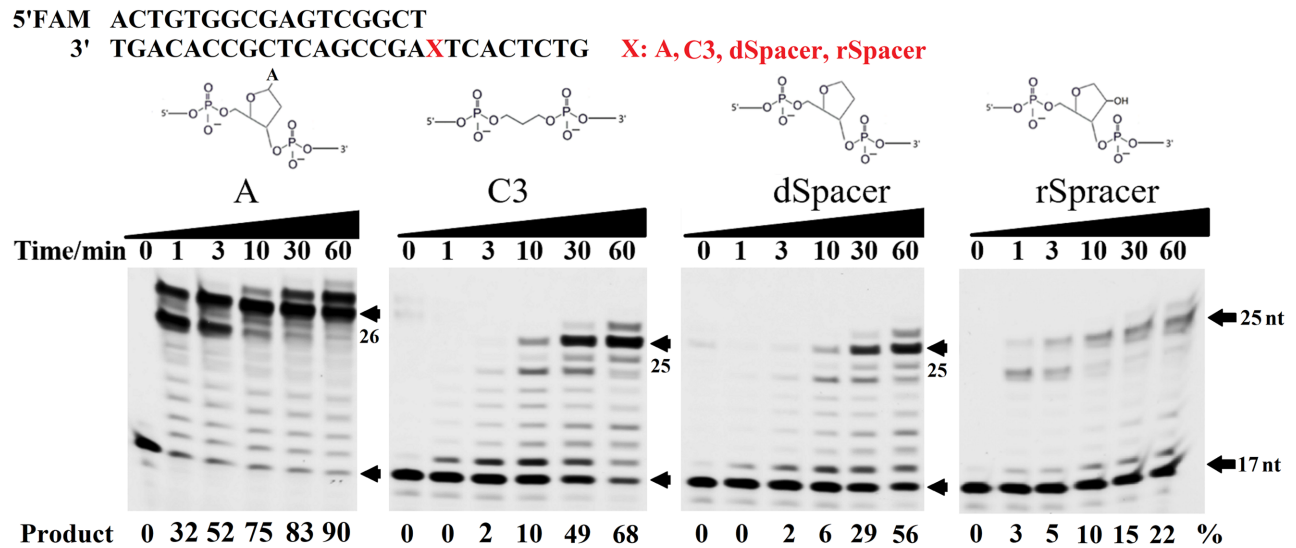
Figure 3. Comparison of the TLS efficiency of dSpacer, rSpacer, and spacer C3 The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating the normal base A or the AP site analogues of dSpacer, rSpacer, and spacer C3. The molecular structures of each spacer are shown above each gel diagram. The primers were annealed to each DNA template strand and extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min. The bands of the 17-nt primer and 25-nt (AP sites) or 26-nt (A base) full-length products are indicated by arrows.

5'FAM ACTGTGGCGAGTCGGCT
3' TGACACCGCTCAGCCGA**X**TCACTCTG   **X: C2, Dual SH**
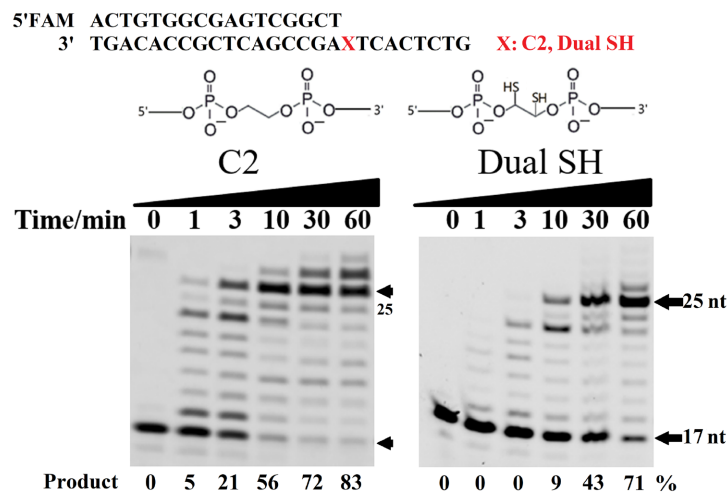
Figure 4. Effect of the dual mercapto groups linked to the alkane chain on TLS The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating the AP site analogues of spacer C2 and of the spacer with dual mercapto groups. The molecular structures of each spacer are shown above each gel diagram. The primers were annealed to each DNA template strand and extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min. The bands of the 17-nt primer and 25-nt full-length products are indicated by arrows.

mercapto groups inhibit Dbh activity. In other words, the introduction of sulphur atoms into the alkane chain spacer further blocks the TLS activity of Dbh.

The distance between the spacer and the 3′-OH of the primer affects TLS by Dhb

Considering that the extension hindrance is severe when the 3′-terminus of the primer is around the spacer, the position of the spacer relative to the 3′-OH of the primer was analyzed for its effect on TLS. Three kinds of spacers, namely, spacers C3, C9 and 18, were used to investigate the effect of the distance of the spacer relative to the 3′-OH of the primer on the TLS activity of Dbh. As shown in Figure 5, when the 3′-OH was around the easily bypassed spacer C3 (position 0), it was difficult to bypass the spacer (Figure

5A, panel 1). The efficiency of bypassing the downstream spacer C3 only slightly increased with the distance between spacer C3 and the 3′-OH of the primer (Figure 5A, panels 2,3), while some extended products were blocked before the spacers (indicated by the red arrows). The results for spacers C9 and 18 were similar to those for spacer C3. When spacer C9 was located at positions −3 and 4 (Figure 5B, panels 1,4), both full-length and short products were more abundant than those obtained when the spacers were located at positions −1 and 0 (Figure 5B, panels 2,3), implying that efficient extension and bypassing the spacer requires the 3′-OH group to be 3–4 nucleotides away from the spacer. However, if spacer C9 was located downstream of the 3′-OH of primer (position 4; Figure 5B, panel 4), the first step of extension was very efficient and was blocked just before spacer (indicated by the red arrows). The sec-
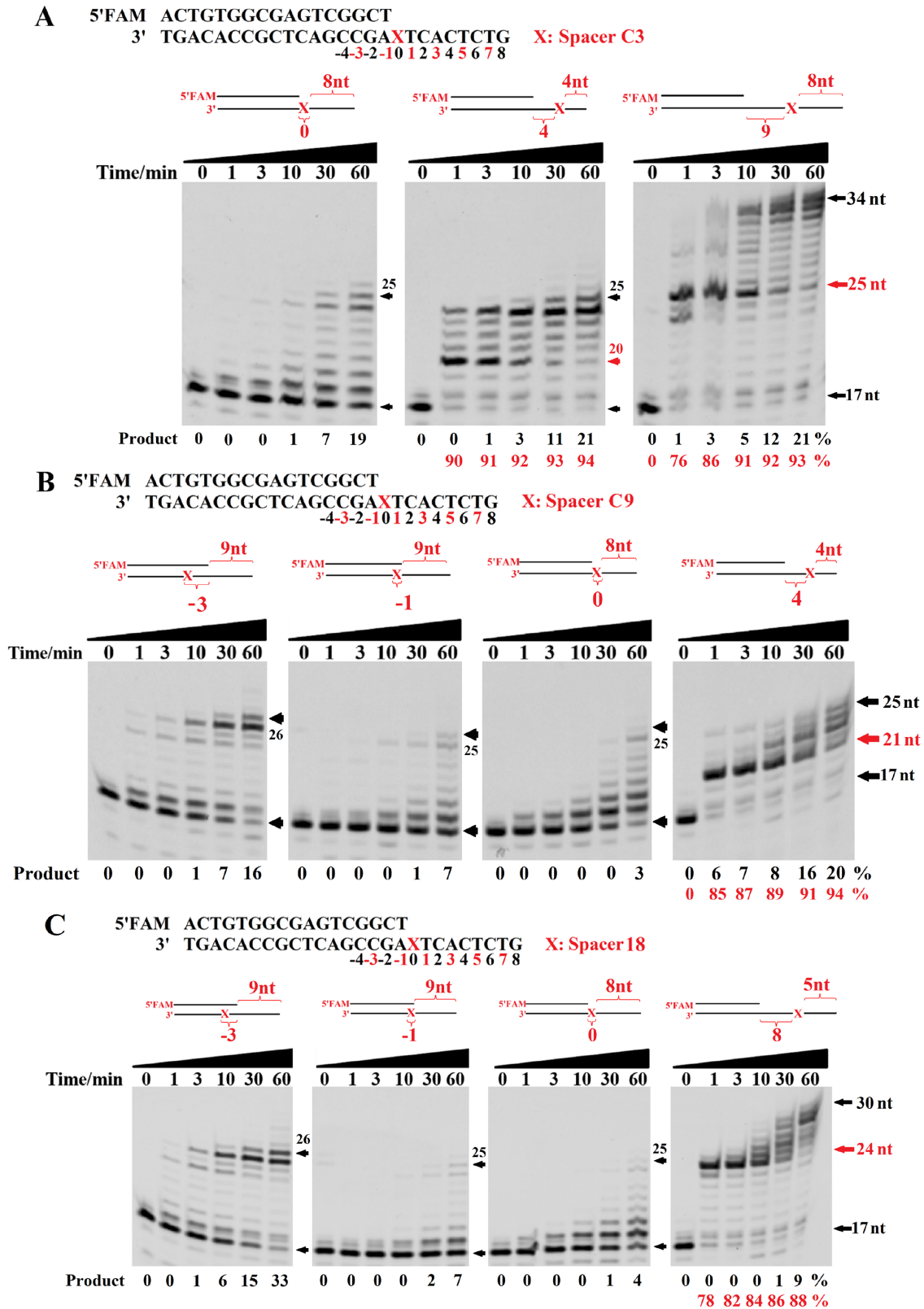
Figure 5. Effect of the distance between the spacer and 3′-terminus of the primer on TLS The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating spacers C3 (A), C9 (B), and S18 (C). The substrates with varied distances between spacers and 3′-teminus of the primer were extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min. The bands of the 17-nt primer and blocked (red arrows) or full-length (25, 26, 30, or 34-nt) products are indicated by arrows. The blocked products were also quantitated by dividing the substrates and shown in red numbers.

ond step of extension, *i.e.*, the bypass of spacer C9, had a lower efficiency, but it was higher than that at positions –1 and 0 (Figure 5B, panels 2,3). For the longest spacer, spacer 18, the closer the 3′-OH was downstream of spacer 18, the more difficult bypassing spacer 18 was (Figure 5C, panels 2,3). If the 3′-OH was far away from the spacer (positions –3 and 8), it was easily extended over the spacers (Figure 5C, panels 1,4). For position 8, two extension steps occurred, and the first step was strongly blocked by spacer 18 (indicated by red arrows in Figure 5C). The bypass step of spacer 18 (Figure 5C, panel 4) was comparable to that upstream of spacer 18 (position –3; Figure 5C, panel 1). In conclusion, when the spacer is adjacent to the primer's 3′-OH, the blocking is stronger, suggesting that the effective binding of the dNTP, template, and 3′-OH of the primer for the formation of a new phosphodiester bond is difficult in the presence of a spacer in the template [21].

The distance between the spacer and the 5′-terminus of the template affects TLS by Dhb

To further investigate the effect of the distance between the spacer and 5′-terminus of the template on the TLS efficiency, DNA template strands containing spacers C3, C9, or 18 with different distances from the 5′ end of the template were used to perform the extension reaction of the primer. The results for spacers C3 and C9 showed that efficient generation of the full-length product required at least four nucleotides downstream of the spacers in the template strand (Figure 6A,B, panels 1,2). During the bypass of the spacer, a large amount of the immediate product (indicated by the red arrows) was blocked by the spacers before extension to the full-length product. If ≤2 nucleotides were located between the spacer and the 5′-terminus of the template strand, the products from the 2-nucleotide template (Figure 6B, panel 3) and 0-nucleotide template (Figure 6B, panel 4) had the same length. In other words, the extension reaction behind the spacer is not dependent on the existence of the 2 nucleotides between the spacer and the 5′-terminus, suggesting that template-independent extension is the main reaction. Since spacer 18 is the most difficult for Dbh to bypass, very little full-length product was generated even when ≥5 nucleotides existed between spacer 18 and the 5′ end of the template strand (Figure 6C, panels 1–3), and a large amount of the product was blocked by the spacer (indicated by the red arrows in Figure 6C). In summary, the longer the spacer is and the closer it is to the 5′-terminus of the template strand, the more difficult the formation of a new phosphodiester bond between the entering nucleotide and the 3′-OH group becomes, which leads to a lower TLS efficiency. However, the extension blockage derived from the shorter distance of spacer to the 5′-terminus of the template strand is relatively less than those derived from the shorter distance of 3′-OH of the primer to the spacer in template.

Discussion

Our previous results showed that Dbh could efficiently bypass the AP site analogue dSpacer [31]. Therefore, we further systematically investigated the TLS properties of Dbh across a series of different AP site analogues. Our results showed that Dbh can bypass various spacers, and the TLS efficiency is related to the spacers' molecular structure, length, and position in the template strand. Among them, spacer length is the most important element for TLS of both alkane chains and polyethylene glycol spacers (Figures 1 and 2). The hindrance of TLS by the spacer becomes more severe when the spacer damage is closer to the 3'-OH group of the extending primer,

which results in a lower TLS efficiency (Figures 5,6). However, as shown in Figure 5A and Figure 6A, the difference in the percentage of bypass by Dbh is not particularly obvious in all three panels. On one hand, this may be due to the different sequence environments surrounding the position of the lesion; on the other hand, the position of the lesion relative to the enzyme changes after each addition of nucleotides during the polymerase replication of the template. For example, after the polymerase Dbh extends the primer by either 4 or 9 nucleotides (in the middle and right panels, respectively), the interaction between Dbh and the lesion will be very similar to the interaction in the substrate shown in the left panel (Figure 5A), and therefore the efficiency of TLS of three substrates is nearly identical.

The cyclization of spacer C3 to dSpacer did not lead to a clear increase in TLS efficiency (Figure 3), implying that the molecular structure of the spacer has a weak effect on TLS. However, the introduction of sulphur atoms (dual mercapto groups) into the carbon chain of spacer C2 strongly decreased the TLS efficiency (Figure 4), which is similar to the inhibition of the AP endonuclease [34]. We speculate that the dual mercapto groups may lead to more harmful interactions with the active centre of Dbh during incorporation of the new deoxynucleotide at the 3′-OH of the primer.

Dbh and Dpo4 are two Y-family polymerases from two closely related archaeal model strains of *S. solfataricus* and *S. acidocaldarius*, respectively, and share 54% amino acid sequence identity [30]. However, they exhibit different enzymatic properties *in vitro* [30]. Dpo4 shows efficient TLS activity for the AP site and thymine dimer damage in the DNA template strand, whereas Dbh does not possess TLS activity towards thymine dimer damage [30]. In addition, Dpo4 tends to produce base substitution mutations, and Dbh tends to produce single base deletion mutations [30,35], which is consistent with our results showing that one base was deleted from the full-length product after bypassing the AP site analogues. We will further elucidate the mechanism of bypassing the various spacers by resolving the crystal structure of the complex of Dbh, a primer, a dNTP, and a template carrying the specific spacer and elucidating the reaction mechanism of the formation of a new phosphodiester bond near the spacer in the template strand.

In addition to base deamination damage, hyperthermophiles also face the stress of spontaneously losing DNA bases, wherein bases are directly removed from the DNA backbone at high temperature to form a base-free site. In addition to the base excision repair pathway for AP site damage, Y-family DNA polymerases play an important role in addressing this damage [36]. Y-family DNA polymerases are relatively conserved in terms of structure and function, but they have different DNA synthesis fidelities and specificities for DNA damage [37]. As error-prone polymerases, Y-family polymerases mainly perform TLS activity when organisms are exposed to conditions that cause severe DNA damage, including AP site damage. The regulation of TLS DNA polymerase in the repair of DNA damage and its coordination with other DNA damage repair partners, especially *in vivo*, are also critical to genome stability. However, there is little knowledge about the *in vivo* functions of Dbh in maintaining genome integrity. Deletion of the *dbh* gene in *S. acidocaldarius* did not cause any obvious growth defects or sensitivity to UV irritation; however, it caused significant changes in the spectrum of spontaneous base mutations and slightly enhanced cisplatin sensitivity [38]. The result showing that the *dbh*⁻ mutant did not exhibit sensitivity to UV irritation is consistent with the inability of Dbh to bypass thymidine dimer damage [19]. The detailed functions
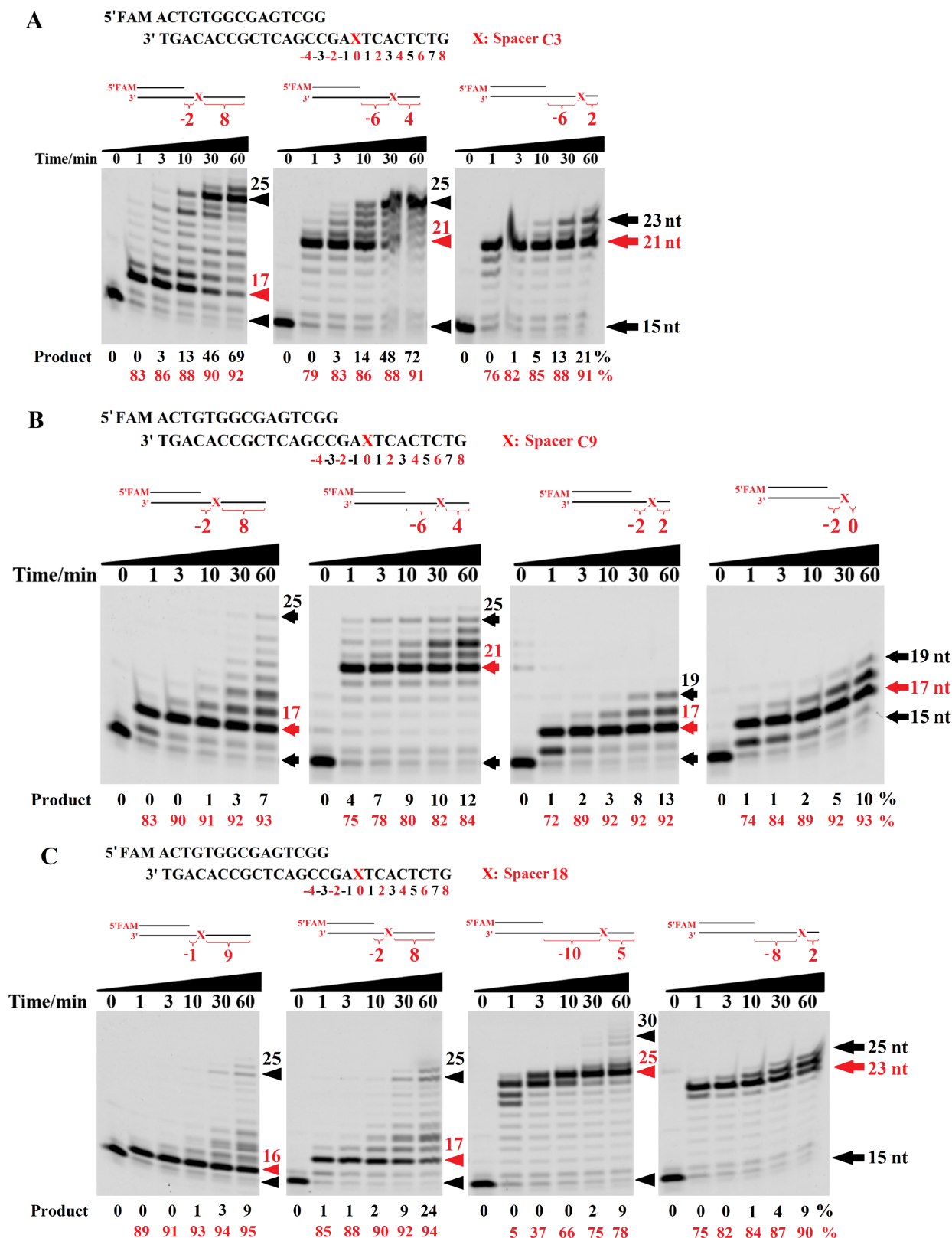
Figure 6. Effect of the distance between the spacer and 5′-terminus of the template strand on TLS The sequences and structures of the DNA substrates are indicated at the top of the diagram, with X indicating spacers C3 (A), C9 (B), and S18 (C). The substrates with varied distances between spacers and 5′-teminus of the template strand were extended by Dbh at 45°C for 0, 1, 3, 10, 30 and 60 min. The bands of the 15-nt primer and blocked (red arrows) or full-length (19, 23, 25, or 30-nt) products are indicated by arrows. The blocked products were also quantitated by dividing the substrates and shown in red numbers.

of the *dbh* gene in maintaining genome integrity will be elucidated by determining the sensitivity of *dbh⁻* or combined mutants of other related genes to additional DNA damage-causing reagents.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. O'Donnell M, Langston L, Stillman B. Principles and concepts of DNA replication in bacteria, archaea, and eukarya. *Cold Spring Harbor Perspectives Biol* 2013, 5: a010108

2. Prakash S, Johnson RE, Prakash L. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annu Rev Biochem* 2005, 74: 317–353

3. Joyce CM, Steitz TA. Function and structure relationships in DNA polymerases. *Annu Rev Biochem* 1994, 63: 777–822

4. Maor-Shoshani A, Hayashi K, Ohmori H, Livneh Z. Analysis of translesion replication across an abasic site by DNA polymerase IV of *Escherichia coli*. *DNA Repair* 2003, 2: 1227–1238

5. Hübscher U, Maga G, Spadari S. Eukaryotic DNA polymerases. *Annu Rev Biochem* 2002, 71: 133–163

6. Sarmiento F, Long F, Cann I, Whitman WB. Diversity of the DNA replication system in the *archaea* domain. *Archaea* 2014, 2014: 1–15

7. Muniandy PA, Liu J, Majumdar A, Liu S, Seidman MM. DNA interstrand crosslink repair in mammalian cells: step by step. *Crit Rev Biochem Mol Biol* 2010, 45: 23–49

8. Sale JE. Competition, collaboration and coordination——determining how cells bypass DNA damage. *J Cell Sci* 2012, 125: 1633

9. Andersen PL, Xu F, Xiao W. Eukaryotic DNA damage tolerance and translesion synthesis through covalent modifications of PCNA. *Cell Res* 2008, 18: 162–173

10. Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL, *et al.* Translesion synthesis: Y-family polymerases and the polymerase switch. *DNA Repair* 2007, 6: 891–899

11. Goodman MF, Woodgate R. Translesion DNA polymerases. *Cold Spring Harbor Perspectives Biol* 2013, 5: a010363

12. Izhar L, Ziv O, Cohen IS, Geacintov NE, Livneh Z. Genomic assay reveals tolerance of DNA damage by both translesion DNA synthesis and homology-dependent repair in mammalian cells. *Proc Natl Acad Sci USA* 2013, 110: E1462–E1469

13. Livneh Z, Ziv O, Shachar S. Multiple two-polymerase mechanisms in mammalian translesion DNA synthesis. *Cell Cycle* 2010, 9: 729–735

14. Yang W, Woodgate R. What a difference a decade makes: insights into translesion DNA synthesis. *Proc Natl Acad Sci USA* 2007, 104: 15591–15598

15. Yang K, Weinacht CP, Zhuang Z. Regulatory role of ubiquitin in eukaryotic DNA translesion synthesis. *Biochemistry* 2013, 52: 3217–3228

16. Pata JD. Structural diversity of the Y-family DNA polymerases. *Biochim Biophys Acta (BBA) - Proteins Proteomics* 2010, 1804: 1124–1135

17. Jarosz DF, Godoy VG, Walker GC. Proficient and accurate bypass of persistent DNA lesions by DinB DNA polymerases. *Cell Cycle* 2007, 6: 817–822

18. Kath JE, Jergic S, Heltzel JM, Jacob DT, Dixon NE, Sutton MD, Walker GC, *et al.* Polymerase exchange on single DNA molecules reveals processivity clamp control of translesion synthesis. *Proc Natl Acad Sci USA* 2014, 111: 7647–7652

19. Sakofsky CJ, Foster PL, Grogan DW. Roles of the Y-family DNA polymerase Dbh in accurate replication of the *Sulfolobus* genome at high temperature. *DNA Repair* 2012, 11: 391–400

20. Ohmori H, Friedberg EC, Fuchs RPP, Goodman MF, Hanaoka F, Hinkle D, Kunkel TA, *et al.* The Y-family of DNA polymerases. *Mol Cell* 2001, 8: 7–8

21. Maxwell BA, Suo Z. Recent insight into the kinetic mechanisms and conformational dynamics of Y-Family DNA polymerases. *Biochemistry* 2014, 53: 2804–2814

22. Sale JE, Lehmann AR, Woodgate R. Y-family DNA polymerases and their role in tolerance of cellular DNA damage. *Nat Rev Mol Cell Biol* 2012, 13: 141–152

23. Reha-Krantz LJ. DNA polymerase proofreading: multiple roles maintain genome stability. *Biochim Biophys Acta (BBA) - Proteins Proteomics* 2010, 1804: 1049–1063

24. Chen L, Brugger K, Skovgaard M, Redder P, She Q, Torarinsson E, Greve B, *et al.* The Genome of *Sulfolobus acidocaldarius*, a model organism of the *crenarchaeota*. *J Bacteriol* 2005, 187: 4992–4999

25. Brock TD, Brock KM, Belly RT, Weiss RL. *Sulfolobus*: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. *Archiv Mikrobiol* 1972, 84: 54–68

26. Wagner M, van Wolferen M, Wagner A, Lassak K, Meyer BH, Reimann J, Albers SV. Versatile genetic tool box for the crenarchaeote *Sulfolobus acidocaldarius*. *Front Microbio* 2012, 3: 214

27. Rivera MC, Lake JA. The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* 2004, 431: 152–155

28. Lindahl T, Nyberg B. Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry* 1974, 13: 3405–3410

29. Sakofsky CJ, Grogan DW. Lesion-induced mutation in the hyperthermophilic archaeon *Sulfolobus acidocaldarius* and its avoidance by the y-family DNA polymerase dbh. *Genetics* 2015, 201: 513–523

30. Boudsocq F, Kokoska RJ, Plosky BS, Vaisman A, Ling H, Kunkel TA, Yang W, *et al.* Investigating the role of the little finger domain of Y-family DNA polymerases in low fidelity synthesis and translesion replication. *J Biol Chem* 2004, 279: 32932–32940

31. Liu XP, Liu JH. The terminal 5′ phosphate and proximate phosphorothioate promote ligation-independent cloning. *Protein Sci* 2010, 19: 967–973

32. Peng L, Xia X, Liu X. Biochemical characterization of translesion synthesis by *Sulfolobus acidocaldarius* DNA polymerases. *Chem Res Chin Univ* 2016, 32: 226–233

33. Shida T, Noda M, Sekiguchi J. Cleavage of single- and double-stranded DNAs containing an abasic residue by *Escherichia coli* exonuclease III (AP endonuclease VI). *Nucleic Acids Res* 1996, 24: 4572–4576

34. Wang WW, Zhou H, Xie JJ, Yi GS, He JH, Wang FP, Xiao X, *et al.* *Thermococcus eurythermalis* endonuclease IV can cleave various apurinic/apyrimidinic site analogues in ssDNA and dsDNA. *Int J Mol Sci* 2019, 20: 69

35. Kokoska RJ, Bebenek K, Boudsocq F, Woodgate R, Kunkel TA. Low fidelity DNA synthesis by a Y family DNA polymerase due to misalignment in the active site. *J Biol Chem* 2002, 277: 19633–19638

36. Ling H, Boudsocq F, Woodgate R, Yang W. Crystal structure of a Y-family DNA polymerase in action. *Cell* 2001, 107: 91–102

37. Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff RV, Walker GC. Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev* 2009, 73: 134–154

38. Miyabayashi H, Jain R, Suzuki S, Grogan DW, Kurosawa N. PolB1 is sufficient for DNA replication and repair under normal growth conditions in the extremely thermophilic crenarchaeon *Sulfolobus acidocaldarius*. *Front Microbiol* 2020, 11: 613375