Processive searching ability varies among members of the gap-filling DNA polymerase X family

Michael J. Howard and Samuel H. Wilson

From the Genome Integrity and Structural Biology Laboratory, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709

Edited by Patrick Sung

DNA repair proteins must locate rare damaged sites within the genome. DNA polymerase β (Pol β), a member of the DNA polymerase X family that is involved in base excision repair, uses a processive hopping search mechanism to locate substrates. This effectively enhances its search footprint on DNA, increasing the probability of locating damaged sites. Processive searching has been reported or proposed for many DNA-binding proteins, raising the question of how widespread or specific to certain enzymes the ability to perform this function is. To provide insight into this question, we compared the ability of three homologous DNA Pol X family members to perform a processive search for 1-nucleotide gaps in DNA using a previously developed biochemical assay. We found that at near-predicted physiological ionic strengths, the intramolecular searching ability of Pol β is at least 4-fold higher than that of Pol µ and ∼2-fold higher than that of Pol α. Pol β also was able to perform intersegmental transfer with the intersegmental searching ability of Pol β being at least 6- and ∼2-fold higher than that of Pols µ and α, respectively. Mutational analysis suggested that differences in the N-terminal domains of these polymerases are responsible for the varying degrees of searching competence. Of note, the differences in processive searching ability observed among the DNA Pol X family members correlated with their proposed biological functions in base excision repair and nonhomologous end joining.

To prevent mutations or cell death, DNA repair proteins must locate rare events of DNA damage among the 6 billion base pairs of the human genome. This can be achieved through a protein-protein recruitment mechanism, post-translational modifications, random 3D diffusion, and/or facilitated diffusion (processive searching). Processive searching occurs when a protein binds nonspecifically to DNA and uses it as a conduit to facilitate substrate site location (1). Processive searching can be further classified by the mechanism the protein uses to diffuse along the DNA. These mechanisms include sliding, hopping, and intersegmental transfer (1, 2). Sliding involves continual contact with a single DNA backbone. In contrast, hopping involves microscopic dissociation and reassociation events that allow the protein to sample both DNA backbones. Both of these intramolecular events are stochastic and effectively serve to increase the DNA-binding footprint of a searching protein on a segment of DNA. Intersegmental transfer is the direct transfer of a protein from one DNA segment to another via a transient microscopic dissociation event or through a bridging intermediate (1, 3). Intersegmental transfer provides a mechanism for global search of DNA as opposed to more local searching observed with hopping and sliding, and it is proposed to be a more efficient global search mechanism than 3D diffusion because it maximizes time on DNA.

Many DNA repair proteins (4–7), transcription factors (8–11), and endonucleases (12–14) have been suggested to use processive searching mechanisms for DNA site location. Descriptions of proteins not using a processive searching mechanism are rare. One such reported example is Escherichia coli RNA polymerase where fluorescent single-molecule studies revealed that this enzyme uses a 3D search mechanism for promoter location as opposed to processive searching (15, 16). However, this study could not rule out short processive searching as the spatial resolution limit of their assays was ∼39 nm (∼115 bp) (16). With the list of proteins that use processive searching growing, questions arise about the specificity of such a process. Indeed, many proteins that bind specific sites in DNA can also bind DNA nonspecifically, a result of overlapping interactions in specific and nonspecific binding modes (17). These observations have led to the hypothesis that many DNA-binding enzymes use processive searching to locate their target sites and that this is a general feature of such proteins (17). To provide further insight into this prevailing view, we have examined the ability of three DNA polymerases to perform a processive search for 1-nt² DNA gaps.

The DNA Pol X family contains four enzymes in humans: Pols β, µ, λ, and terminal deoxynucleotidyltransferase (TdT) (18). DNA Pol β is critical in the BER pathway and has been demonstrated to perform a processive search (19). DNA Pols µ

²The abbreviations used are: nt, nucleotide; Pol, DNA polymerase; TdT, terminal deoxynucleotidyltransferase; BER, base excision repair; NHEJ, nonhomologous end joining; BRCT, BRCA1 C terminus; Fₚ, fraction processive; AAG, alkyladenine DNA glycosylase; E, efficiency; FAM, 6-fluorescein amide; dGPPp, 2'-deoxyguanosine 5’-(α,β-methylene)triphosphate.
and λ are involved in nonhomologous end joining (NHEJ) with Pol λ having a backup role in BER (20, 21). Pols μ and λ are structurally similar to Pol β except for additional domains on their N termini (Fig. 1); both contain a BRCT domain and Pol λ a serine/proline-rich region, that is proposed to be a site for post-translational modifications (22). The BRCT domains of these enzymes are important for protein-protein interactions during NHEJ (23, 24), suggesting that their recruitment to damage is dependent on this domain. Pols β, μ, and λ can catalyze nucleotide incorporation into 1-nt-gapped DNA, whereas TdT does not (24). Additionally, Pols μ and λ have varying template dependences (24). For instance, Pols μ and λ can both catalyze nucleotide insertion on DNA substrates that contain breaks within the template backbone, whereas Pol β has low or no activity on such a substrate (24). These differences likely reflect the substrates encountered during their respective biological roles.

We previously demonstrated that Pol β uses an ionic strength-dependent hopping search mechanism and now extend these studies to show that Pol β can perform intersegmental transfer. Comparative studies with Pols μ and λ demonstrate that Pol β displays the highest processive searching ability among these Pol X family members. The differing levels of searching ability may reflect the biological roles these polymerases play within the cell and suggest that the ability of Pol β to search DNA was selected for during its evolution.

**Results**

**Characterization of single nucleotide gap filling catalyzed by the X family polymerases**

Because the processive search assay is based on nucleotide insertion into 1-nt-gapped DNA, the ability of the three polymerases to catalyze this reaction was first examined (Fig. 1C).

With saturating concentrations of nucleotide, the single-turnover rate constants ($k_{pol}$) were similar for Pol β ($2.9 \text{ s}^{-1}$) and Pol μ ($1.4 \text{ s}^{-1}$), whereas the $k_{pol}$ for Pol λ ($0.17 \text{ s}^{-1}$) is ~10-fold lower. These studies confirm single-nucleotide insertion activity for these enzymes and provide a basis for interpreting the following processive search results.

**A gradient of processive searching ability for the Pol X family**

Using our standard processive assay substrate (P20), where two 1-nt gaps on the same DNA strand are separated by 20 bp, the ability of Pols μ and λ to perform a processive search was examined (Fig. 2). Processive search assays were performed under steady-state conditions, and the initial velocities for distributive and processive products were extracted to calculate fraction processive ($F_p$) values. The $F_p$ value is the probability of an enzyme to perform a productive search, i.e. the ability to locate and catalyze product formation at both substrate sites within a single DNA-binding event (5, 19). Representative gels for processive assays with Pols β and μ are shown in Fig. 3A. Pol β was at least 4- and ~2-fold more processive than Pols μ and λ, respectively, at 100 mM ionic strength (Fig. 3C and supplemental Table S1). These data indicate that there is a spectrum of searching ability with the Pol X family members.

To determine whether the additional N-terminal domains of Pols μ and λ could account for the observed differences in searching ability, as compared with Pol β, truncated forms of these enzymes were used in the processive search assay. These truncations maintain the 8-kDa and polymerase domains only (Fig. 1A). Removal of the BRCT domain of Pol μ ($\Delta 131$Pol μ) does not affect its searching ability (Fig. 3C). Interestingly, removal of the N-terminal domains of Pol λ ($\Delta 241$Pol λ) significantly but modestly increases $F_p$ (1.6-fold) as compared with full-length Pol λ (Fig. 3C and supplemental Table S1). These
data suggest that the additional domains on Pol λ inhibit its processive searching ability, whereas the presence of the BRCT domain in Pol μ has no effect.

**Pol μ can perform intersegmental transfer**

Facilitated diffusion includes mechanisms of hopping, sliding, and intersegmental transfer (1, 2). We previously demonstrated that Pol β can perform an intramolecular hopping mechanism, but its ability to perform intersegmental transfer is unknown. To determine whether Pol β can perform intersegmental transfer, we used a substrate that was previously utilized to characterize the intersegmental transfer ability of the DNA glycosylase AAG (3). This intersegmental transfer substrate contains two DNA duplexes, each containing a 1-nt gap, that are covalently linked through varying PEG linker lengths. This allows for the close positioning of two DNA duplexes with a predicted effective concentration in the low millimolar range (25, 26). Two intersegmental transfer substrates were used: IS4 and IS8, containing four and eight PEG-6 linkers, respectively, where the 6 indicates the number of ethylene glycol units (Fig. 4A). As shown in Fig. 4B, Pol β catalyzed a significant amount of processive product with this substrate, indicating that Pol β can perform intersegmental transfer. Increasing the PEG-6 linker length from four to eight units has no significant effect on $F_p$ (Fig. 5B and supplemental Table S3). This lack of dependence is most likely due to the short persistence length of PEG (~4 Å) (27), and this is consistent with the behavior of the DNA glycosylase AAG (3). The ionic strength dependence of the fraction processive with the IS4 substrate was indistinguishable from that of the P20 intramolecular substrate (Fig. 5A and supplemental Table S2). We previously showed that mutation of three lysines in the 8-kDa lyase domain of Pol β to alanine (K35A, K68A, and K72A; referred to as KΔ3A) significantly reduced processive searching with the P20 intramolecular substrate (19). This mutant similarly disrupted the ability of Pol β to perform intersegmental transfer (Fig. 5B). Taken together, these data demonstrate that Pol β can perform intersegmental transfer and that this process shares a similar ionic strength dependence with the intramolecular hopping mechanism.

Next it was determined whether Pols μ and λ can perform intersegmental transfer. As shown in Fig. 6B, full-length Pols μ and λ are at least 6- and ~3-fold less processive than Pol β, respectively (supplemental Table S4). Overall the $F_p$ values obtained with the intersegmental transfer substrate mirror the $F_p$ values obtained with the P20 hopping substrate, consistent with the proposal that hopping and intersegmental transfer share similar mechanisms (see “Discussion”).

**Determination of nucleotide insertion efficiency values for Pols β, μ, and λ**

Processive searching, as measured here, is dependent on DNA scanning and nucleotide incorporation once a gap is encountered (i.e. product formation) (19). It is possible that a Pol may scan to a second site but dissociate before catalyzing nucleotide insertion, which would lead to a decrease in $F_p$. The efficiency ($E$) is the probability of a Pol to catalyze nucleotide insertion versus dissociating from the 1-nt-gapped DNA substrate (6). To determine whether DNA scanning or efficiency drive the differences in the $F_p$ values observed among the Pols, we measured the efficiency of nucleotide insertion (Fig. 7). The efficiency values for Pol β and λ are high, 0.78 and 0.93, respectively, indicating that these Pols have a high probability of inserting a nucleotide once a gap is encountered. The efficiency for Pol μ is ~2-fold lower, 0.39.

**Discussion**

A fundamental problem facing any DNA repair enzyme is the task of locating its substrate. Facilitated diffusion is one possible solution to this search problem. In this study, we sought to determine whether the searching ability of Pol β is unique to this member of the human Pol X family or whether it is a shared feature among these polymerases. Pol X family members exhibit a spectrum of processive searching abilities for DNA gaps. Pol β demonstrates the highest $F_p$ values in contrast to values ~2-fold lower and at least 4-fold lower for Pol λ and Pol μ, respectively, as compared with Pol β. Because Pols β, μ, and λ can all perform DNA gap filling, these data may suggest that the searching ability of Pol β has evolved specifically for substrate location. Examples in the literature of DNA repair enzymes not performing processive searching are rare, leading to the view that many, if not all, DNA repair enzymes use processive searching for substrate localization. Here we show that even among homologous enzymes there are varying degrees of processive searching.

It is important to discuss the interpretation of low $F_p$ values, such as the ones observed with Pol μ. Using our processive assay, the lowest $F_p$ value observed was 0.05, determined at 150 mM ionic strength with the KΔ3A Pol β mutant (19). Similar low $F_p$ values have been obtained at high ionic strengths (0.08)
**Figure 3. Intramolecular searching abilities of DNA Pols β, μ, and λ.**

A, representative gels of processive assays performed with 500 nM P20 substrate, 1 nM Pol β, and 3 nM Pols μ and λ at 100 mM ionic strength. Time points are indicated at the top of the gels. S indicates substrate bands, P indicates processive product, and D represents distributive products. B, representative time course for Pol μ-catalyzed nucleotide insertion on P20 substrate, resulting in $F_p$ and $k_{cat}$ values of 0.1 and 0.01 s$^{-1}$, respectively. C, bar graph representing the fraction processive values for full-length and truncated forms of Pol β, μ, and λ enzymes (supplemental Table S1). The error bars represent the S.D. from at least two experiments.

**Figure 4. Pol β can perform intersegmental transfer.**

A, the intersegmental substrate is composed of two DNA duplexes, each containing a 1-nt gap (indicated by circle), attached through varying PEG-6 unit lengths, termed IS4 and IS8 for four and eight PEG-6 units, respectively. B, representative gel of a processive assay performed with Pol β and the intersegmental transfer substrate (IS8). P indicates processive product, and D represents distributive products. C, representative plot (quantification of B) of intersegmental transfer processive assay ($F_p = 0.45$).
and with a site spacing of 80 bp (0.06). It is unlikely that the processive hopping mechanism of Pol β could persist at high ionic strengths; thus these low values likely represent the background of the assay (0.05–0.08). The background arises from the enzyme rebinding to the intermediate substrates to form the processive product in a distributive manner. Similar background behavior has been observed with AAG (5). Additionally, because the \( k_{cat} \) values of Pols \( \mu \) and \( \lambda \) are significantly lower than that of Pol β, the concentrations of Pols \( \mu \) and \( \lambda \) used in the processive assays described here were \(~3\)-fold higher than for Pol β (supplemental Table S1). This increases the probability of rebinding to intermediates. For transparency, we have chosen to represent the data as obtained without correction. We propose that the \( F_p \) values observed with Pol \( \mu \) likely represent background activity, and therefore Pol \( \mu \) is most likely not processive under these conditions. However, we cannot rule out the possibility that Pol \( \mu \) uses some amount of processive searching inside the cell because our assay conditions are probably not mimicking such an environment. For instance, the addition of crowding agents can increase processive searching (28). But if we assume that these effects would scale with the \(~3\)-fold differences we observed between the polymerases, then it is reasonable to propose that Pol β is more processive than Pols \( \mu \) and \( \lambda \).

Figure 5. Pol β intersegmental transfer ionic strength and PEG linker length dependence. A, the ionic strength dependence of intersegmental transfer (IS4) is indistinguishable from the hopping substrate (P20) (supplemental Table S2). The ionic strength data for P20 is taken from Ref. 19. B, variation of the PEG linker length with the intersegmental transfer substrate has no effect on the fraction processive (supplemental Table S3). The KΔ3A mutant reduces intersegmental transfer ability of Pol β. The error bars represent the S.D. from at least two assays.

Figure 6. Comparison of intersegmental transfer abilities of Pol X family members. A, gels representing processive assays performed with \( \Delta 131 \)Pol μ and \( \Delta 241 \)Pol λ truncated enzymes and the intersegmental transfer substrate (IS8). Assays were performed as described in Fig. 4 at 100 mM ionic strength. \( S \) indicates substrate bands, \( P \) indicates processive product, and \( D \) represents distributive products. B, bar graph representing the \( F_p \) values obtained with the indicated enzyme and the IS8 substrate (supplemental Table S4). The error bars represent the mean and S.D. from at least two assays except for the values reported for Pol μ.
The observed $F_p$ value is dependent on the searching ability and the efficiency of nucleotide insertion (6). Measurement of the efficiency values for these Pols reveals that the low $F_p$ values observed with Pol λ, as compared with Pol β, are due to a decrease in searching ability as Pol λ has a high efficiency value (0.93). The decrease in the $F_p$ value observed with Pol μ derives from decreases in both searching ability and efficiency. It may be possible that protein–protein interactions (i.e. Ku70/80) increase the efficiency for Pol μ. In this case, the $F_p$ may increase to 0.3 from 0.12 for the P20 intramolecular substrate at 100 mM ionic strength. Similarly, increasing the efficiency to 1.0 for Pol λ would only increase the $F_p$ to 0.24 from 0.22 for the same substrate and conditions. Thus, even if the efficiency was increased to 1.0 for both Pol μ and λ, the $F_p$ would still be ~2-fold lower than the $F_p$ value for Pol β.

The gradient of searching abilities observed correlates with the proposed biological functions of these polymerases. Pol β is involved in BER; Pol μ and Pol λ are involved in NHEJ with Pol λ having a backup role in BER (20, 21). Pol β has no dedicated protein–protein interaction domain, whereas Pol μ and λ have BRCT domains. The BRCT domains of Pol μ and λ are required for polymerase activity in in vitro NHEJ assays (23). These results are consistent with the idea that Pols μ and λ are localized to double strand breaks via interaction with the DNA-bound Ku70/80 complex through their BRCT domains (Fig. 8B). Thus, the selective pressure for the ability to perform a processive search may be absent in the Pols involved in NHEJ. In the case of Pol λ, which has a backup role in BER, the selective pressure of having a searching ability may be increased, consistent with its higher $F_p$ values compared with Pol μ.

We previously demonstrated that the positively charged 8-kDa domain of Pol β is instrumental for searching and proposed a model where this domain makes nonspecific electrostatic interactions with DNA during the search process (19). The theoretical isoelectric points (pI) of the 8-kDa domains of Pols β, μ, and λ are 9.7, 5.7, and 9.4, respectively. Thus, our model predicts the searching ability of Pol μ to be significantly decreased as compared with Pol β. Indeed, the $F_p$ value of Pol μ with the P20 hopping substrate is at least 4-fold lower than that of Pol β (Fig. 3C). The 8-kDa domain of Pol λ has a pI similar to that of the 8-kDa domain of Pol β; however, the $F_p$ value for full-length Pol λ is ~2-fold lower than that for Pol β. This lower than expected value arises from the additional N-terminal domains because Δ242Pol λ has an $F_p$ value closer to that of Pol β. Therefore, we suggest that the N-terminal domains of Pol λ decrease its ability to search (~2-fold). The observed increase in processivity upon truncation of Pol λ could be due to an
Figure 8. Proposed models of DNA Pol X localization to respective substrates. A, processive searching model for Pol β. After catalyzing product (forming a nick), a series of steps occurs for productive processive searching: 1) microdissociation from product; 2) reassociation to undamaged DNA or direct transfer to an adjacent proximal DNA; 3) dissociation from undamaged DNA (steps 2 and 3 repeated give rise to a hopping mechanism); and 4) association to substrate. B, proposed model for Pol μ and λ localization to a double strand (Ds) break during NHEJ. Pols μ and λ are recruited to double strand breaks through the Ku70/80 complex by their BRCT domains.

enhanced efficiency of nucleotide insertion, or the additional domains destabilize nonspecific DNA binding during the search process. These data further support the importance of the positively charged 8-kDa domain in searching.

Intersegmental transfer is the direct transfer of a protein from one DNA segment to another. There are two models for how this can occur: the bridging and the jumping mechanisms (3). In the bridging model, the protein transfers to another DNA through an intermediate where the protein is simultaneously bound to both DNA segments. This mechanism has been proposed for proteins that have multiple DNA-binding sites, either through having multiple DNA-binding domains or through dimerization (8–10, 29–35). During the microscopic dissociation event, the protein can either reassociate with the same strand (a hop (intramolecular)) or transfer to a neighboring DNA (a jump (intermolecular)). Our results are best explained by a jumping model, but we cannot rule out a bridging mechanism. Indeed, these two models are not mutually exclusive as the Oct-1 transcription factor seems to use both mechanisms (8). For Pol β, the observation of a similar ionic strength dependence between the hopping substrate (P20) and the intersegmental transfer substrate (IS4) is evidence supporting a jumping mechanism (Fig. 5A). A similar dependence on ionic strength is consistent with the proposal of a hop and a jump arising from the same intermediate, a microdissociated protein.

In conclusion, not all DNA-associated enzymes perform processive searching, suggesting that selective pressure to do so was present during the evolution of Pol β. The processive searching mechanism of Pol β is a multistep process that encompasses these minimal steps: 1) microdissociation from product, 2) reassociation to undamaged DNA, 3) microdissociation from undamaged DNA, and 4) association to substrate, which includes a conformational change that enhances binding affinity for substrate (Fig. 8A). Cycling between steps 2 and 3 results in a hopping mechanism. During a microdissociated state, the protein can either reassociate with the same DNA (a hop) or bind another nearby DNA (a jump). Thus, intersegmental transfer is a hop that has been coopted by a closely positioned DNA neighbor. This allows Pol β to escape a redundant search on a single DNA segment while optimizing its search time on DNA.

Experimental procedures

Enzymes

Enzymes were prepared as described previously (36–38). The extinction coefficients used to calculate the concentration of the proteins are 23,380, 47,440, 35,000, 57,870, and 36,900 M⁻¹ cm⁻¹ for Pol β, Pol μ, Δ131Pol μ (Δ1–131), Pol λ, and Δ241Pol λ (Δ1–241), respectively.

Substrates

All oligos were from Integrated DNA Technologies, Inc. The P20 substrate was the same as described previously (19). Intersegmental transfer substrates contained five oligos designated IS1, IS2_4, IS2_8, IS3, IS4, and IS5. The IS2_4 and IS2_8 oligos contained four and eight PEG-6 units, respectively (IS4 and IS8 contained 24 and 48 ethylene glycol units, respectively). DNA sequences are provided in the supplemental material. Substrates were annealed with equal molar ratios in a PCR machine.

Single-turnover nucleotide insertion

Reactions were performed with a quench-flow apparatus. Reaction conditions included 50 mM Tris, pH 7.4, 100 mM KCl, 5 mM MgCl₂, 1 mM DTT, 10% glycerol, and 0.1 mg/ml BSA at 37 °C. Reactions initiated by mixing 100 nM DNA and 100 µM dCTP for Pol β and Pol λ reactions and 2 mM dCTP for Pol μ reactions in reaction buffer with a 1 µM concentration of the indicated polymerase equilibrated in reaction buffer. Reactions were stopped by addition of 100 mM EDTA. Samples were then separated on a 22% gel and quantified as described previously (19).

Processive assays

Assays were performed as described previously (19). Briefly, reaction mixes contained 1 nM Pol β, 3 nM Pol μ or λ, 500 nM processive substrate, 50 µM dCTP, 50 mM Tris-HCl, pH 7.4, KCl at the concentration indicated, 5 mM MgCl₂, 1 mM DTT,
Trapped reactions are fit to a burst equation (39) (Pols efficiencies were determined for both distributive and processive searching by gap-filling DNA polymerases. Reaction time points were quenched with EDTA, and products and revised the paper. All authors approved the final version of the manuscript.

Efficiency measurements

We previously used a pulse-chase partition assay to measure the probability of Pol β to catalyze nucleotide insertion versus dissociation from the DNA substrate (19). This probability is referred to as the efficiency (6, 39, 40). Previously, these measurements were performed on a KinTek quench-flow apparatus and required a large volume of concentrated trap (1-nt-gapped DNA). We have simplified this assay by manually mixing the binary complex with a solution containing both dCTP and trap. This assumes that nucleotide binds faster than the dissociation of the Pol from the DNA. Determining the efficiency values by either method results in comparable values (for pulse chase, $E = 0.74$, and manually, $E = 0.78$ for Pol β). In the manual mixing partition assay, a binary complex containing a 400 nM concentration of the indicated Pol and 200 nM FAM-DNA substrate (1-nt gap with a templating G) was mixed with an equal volume of 100 μM dCTP and 2 μM dGPcPP (indicated as dCTP) or 100 μM dCTP, 4 μM trap DNA, and 2 μM dGPcPP (indicated as dCTP + Trap). Reaction conditions included 50 mM Tris, pH 7.4, 50 mM KCl, 5 mM MgCl₂, 10% glycerol, 1 mM DTT, and 0.1 mg/ml BSA at 37 °C. At the indicated time points, a sample of the reaction was removed and analyzed as described for the single-turnover reactions. The trap was made more effective by adding nonhydrolyzable nucleotide (dGPcPP; Jena Biosciences), which is the correct insertion for the trap DNA (templatting C), to the reaction mixtures. The dCTP reactions are fit to a single-exponential equation (Pols μ and λ) or a line (Pol β). Trapped reactions are fit to a burst equation (39) (Pols μ and λ) or a line (Pol β). A trap control reaction was performed by preincubating the indicated binary complex with trap DNA and 2 μM dGPcPP for 5 min at 37 °C before initiating the reaction by addition of dCTP to a final concentration of 50 μM. The efficiency was calculated by dividing the amplitude of the burst phase or the y intercept of the dCTP + Trap reaction by the amplitude or the y intercept of the dCTP reaction (39).

Acknowledgments—We thank Lars Pedersen and Andrea Moon of the X-ray crystallography core laboratory at National Institute of Environmental Health Sciences and Kasia Bebenek for the generous gift of purified Pols μ and λ. We also thank David Shock and William Beard for general advice on designing and performing kinetic assays.

References

1. Berg, O. G., Winter, R. B., and von Hippel, P. H. (1981) Diffusion-driven mechanisms of protein translocation on nucleic acids. 1. Models and theory. Biochemistry 20, 6929–6948
2. Halford, S. E., and Marko, J. F. (2004) How do site-specific DNA-binding proteins find their targets? Nucleic Acids Res. 32, 3040–3052
3. Hedglin, M., Zhang, Y., and O’Brien, P. J. (2013) Isolating contributions from intersegmental transfer to DNA searching by alkyladenine DNA glycosylase. J. Biol. Chem. 288, 24550–24559
4. Carey, D. C., and Strauss, P. R. (1999) Human apurinic/apyrimidinic endonuclease is processive. Biochemistry 38, 16553–16650
5. Hedglin, M., and O’Brien, P. J. (2008) Human alkyladenine DNA glycosylase employs a processive search for DNA damage. Biochemistry 47, 11434–11445
6. Porecha, R. H., and Stivers, J. T. (2008) Uracil DNA glycosylase uses DNA hopping and short-range sliding to trap extrahelical uracils. Proc. Natl. Acad. Sci. U.S.A. 105, 10791–10796
7. Rowland, M. M., Schonhoff, J. D., McKibbin, P. L., David, S. S., and Stivers, J. T. (2014) Microscopic mechanism of DNA damage searching by hOGG1. Nucleic Acids Res. 42, 9295–9303
8. Doucette, M., and Clore, G. M. (2008) Global jumping and domain-specific intersegment transfer between DNA cognate sites of the multidomain transcription factor Oct1. Proc. Natl. Acad. Sci. U.S.A. 105, 13871–13876
9. Iwahara, J., and Clore, G. M. (2006) Direct observation of enhanced translational exchange between homeodomain DNA and nucleic acids. J. Am. Chem. Soc. 128, 404–405
10. Takayama, Y., and Clore, G. M. (2011) Intracellular- and intermolecular-translocation of the bi-domain transcription factor Oct1 characterized by liquid crystal and paramagnetic NMR. Proc. Natl. Acad. Sci. U.S.A. 108, E169–E175
11. Esadze, A., Kenna, C. A., Kolomeisky, A. B., and Iwahara, J. (2014) Positive and negative impacts of nonspecific sites during target location by a sequence-specific DNA-binding protein: origin of the optimal search at physiological ionic strength. Nucleic Acids Res. 42, 7039–7046
12. Gowers, D. M., Wilson, G. G., and Halford, S. E. (2005) Measurement of the contributions of 1D and 3D pathways to the translocation of a protein along DNA. Proc. Natl. Acad. Sci. U.S.A. 102, 15883–15888
13. Stanford, N. P., Szczelkun, M. D., Marko, J. F., and Halford, S. E. (2000) One- and three-dimensional pathways for proteins to reach specific DNA sites. EMBO J. 19, 6546–6557
14. Terry, B. J., Jack, W. E., and Modrich, P. (1985) Facilitated diffusion during catalysis by EcoRI endonuclease. Nonspecific interactions in EcoRI catalysis. J. Biol. Chem. 260, 13130–13137
15. Friedmann, L. J., Mumma, J. P., and Gelles, I. (2013) RNA polymerase approaches its promoter without long-range sliding along DNA. Proc. Natl. Acad. Sci. U.S.A. 110, 9740–9745
16. Wang, F., Redding, S., Finkelstein, I. J., Gorman, J., Reichman, D. R., and Greene, E. C. (2013) The promoter-search mechanism of Escherichia coli RNA polymerase is dominated by three-dimensional diffusion. Nat. Struct. Mol. Biol. 20, 174–181
17. Redding, S., and Greene, E. C. (2013) How do proteins locate specific targets in DNA? Chem. Phys. Lett. 570, 10.1016/j.cplett.2013.03.035
18. Bienstock, R. J., Beard, W. A., and Wilson, S. H. (2014) Phylogenetic analysis and evolutionary origins of DNA polymerase X-family members. DNA Repair 22, 77–88
19. Howard, M. J., Rodriguez, Y., and Wilson, S. H. (2017) DNA polymerase B uses its lyase domain in a processive search for DNA damage. Nucleic Acids Res. 45, 3822–3832
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20. Braithwaite, E. K., Kedar, P. S., Stumpo, D. J., Bertocci, B., Freedman, J. H., Samson, L. D., and Wilson, S. H. (2010) DNA polymerases β and λ mediate overlapping and independent roles in base excision repair in mouse embryonic fibroblasts. PLoS One 5, e12229

21. Braithwaite, E. K., Prasad, R., Shuck, D. D., Hou, E. W., Beard, W. A., and Wilson, S. H. (2005) DNA polymerase λ mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts. J. Biol. Chem. 280, 18469–18475

22. Frouin, I., Touelle, M., Ferrari, E., Shevelev, I., and Hübscher, U. (2005) Phosphorylation of human DNA polymerase λ by the cyclin-dependent kinase Cdk2/cyclin A complex is modulated by its association with proliferating cell nuclear antigen. Nucleic Acids Res. 33, 5354–5361

23. Mahajan, K. N., Nick McElhinny, S. A., Mitchell, B. S., and Ramsden, D. A. (2002) Association of DNA polymerase μ (pol μ) with Ku and ligase IV: role for pol μ in end-joining double-strand break repair. Mol. Cell. Biol. 22, 5194–5202

24. Nick McElhinny, S. A., Havener, J. M., Garcia-Diaz, M., Juárez, R., Bebenek, K., Kee, B. L., Blanco, L., Kunkel, T. A., and Ramsden, D. A. (2005) A gradient of template dependence defines distinct biological roles for family X polymerases in nonhomologous end joining. Mol. Cell. Biol. 25, 123–128

25. Tian, L., and Heyduk, T. (2009) Bivalent ligands with long nanometer-scale flexible linkers. Biochemistry 48, 264–275

26. Krishnamurthy, V. M., Semetey, V., Bracher, P. J., Shen, N., and Whitesides, G. M. (2007) Dependence of effective molarity on linker length for an intramolecular protein-ligand system. J. Am. Chem. Soc. 129, 1312–1320

27. Kienberger, F., Pastushenko, V. P., Gerald, K., Gruber, H. J., Riener, C., Schindler, H., and Hinterdorfer, P. (2000) Static and dynamical properties of single poly(ethylene glycol) molecules investigated by force spectroscopy, Single Molecules 1, 123–128

28. Cravens, S. L., Schonhoft, J. D., Rowland, M. M., Rodriguez, A. A., Anderson, B. G., and Stivers, J. T. (2015) Molecular crowding enhances facilitated diffusion of two human DNA glycosylases. Nucleic Acids Res. 43, 4087–4097

29. Fried, M. G., and Crothers, D. M. (1984) Kinetics and mechanism in the reaction of gene regulatory proteins with DNA. J. Mol. Biol. 172, 263–282

30. Ruusala, T., and Crothers, D. M. (1992) Sliding and intermolecular transfer of the lac repressor: kinetic perturbation of a reaction intermediate by a distant DNA sequence. Proc. Natl. Acad. Sci. U.S.A. 89, 4903–4907

31. Lieberman, B. A., and Nordeen, S. K. (1997) DNA intersegment transfer, how steroid receptors search for a target site. J. Biol. Chem. 272, 1061–1068

32. Kozlov, A. G., and Lohman, T. M. (2002) Kinetic mechanism of direct transfer of Escherichia coli SSB tetramers between single-stranded DNA molecules. Biochemistry 41, 11611–11627

33. Wentzell, L. M., and Halford, S. E. (1998) DNA looping by the Sfi I restriction endonuclease. J. Mol. Biol. 281, 433–444

34. Gorman, J., Wang, F., Redding, S., Plys, A. J., Fazio, T., Wind, S., Alani, E. E., and Greene, E. C. (2012) Single-molecule imaging reveals target-search mechanisms during DNA mismatch repair. Proc. Natl. Acad. Sci. U.S.A. 109, E3074–E3083

35. Schmidt, H. G., Sewitz, S., Andrews, S. S., and Lipkow, K. (2014) An integrated model of transcription factor diffusion shows the importance of intersegmental transfer and quaternary protein structure for target site finding. PLoS One 9, e108575

36. Beard, W. A., and Wilson, S. H. (1995) Purification and domain-mapping of mammalian DNA polymerase δ. Methods Enzymol. 262, 98–107

37. Moon, A. F., Garcia-Diaz, M., Bebenek, K., Davis, B. J., Zhong, X., Ramsden, D. A., Kunkel, T. A., and Pedersen, L. C. (2007) Structural insight into the substrate specificity of DNA polymerase μ. Nat. Struct. Mol. Biol. 14, 45–53

38. Garcia-Diaz, M., Bebenek, K., Krahn, J. M., Blanco, L., Kunkel, T. A., and Pedersen, L. C. (2004) A structural solution for the DNA polymerase λ-dependent repair of DNA gaps with minimal homology. Mol. Cell 13, 561–572

39. Zhang, Y., and O’Brien, P. J. (2015) Repair of alkylation damage in eukaryotic chromatin depends on searching ability of alkylation DNA glycosylase. ACS Chem. Biol. 10, 2606–2615

40. Hendershot, J. M., and O’Brien, P. J. (July 26, 2017) Search for DNA damage by human alkyladenine DNA glycosylase involves early intercalation by an aromatic residue. J. Biol. Chem. 292, 16070–16080