Supporting Information

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SI Discussion.

The similarities between polymerase and histidinol phosphatase (PHP) domains and endonuclease IV (EndoIV) could be extended to recognition and binding to DNA. Thus, EndoIV binding to DNA is mediated by five protruding R loops, forming a groove of 10–20-Å width with the catalytic core located at the bottom, which makes the protein ideally suited for binding DNA (1). Once bound to DNA, EndoIV scans for the presence of abasic sites. DNA damage detection proceeds by insertion of residues located at the R loops into the DNA base stack through the minor groove. More specifically, residues Arg37 (R-loop 2) and Tyr72 (R-loop 3) stack with the base pair 3′ and 5′ to the abasic site, respectively (2) (see Fig. S1*B*). In addition to its role in flipping the sugar-phosphate backbone at the apurinic/apyrimidinic (AP) site, the aromatic group of EndoIV Tyr72 controls the active site hydrophobic environment to allow catalysis. Modeling of $PolX_{Bs}$ PHP domain on the recently reported $PolX_{Dr}$ structure (3) also shows multiple loops surrounding the catalytic core that, by analogy, could be involved in the recognition and binding of the AP site (see Fig. S1*B*). Residues Arg474 and Phe440, highly conserved in the bacterial/archaeal PHP domain of PolX members (4, 5) and placed at the apex of loops $\beta_4\alpha_4$ and $\beta_5\alpha_5$, respectively (3), could be playing a similar function to the above mentioned EndoIV residues.

PHP domains are present also in replicative pol III α-subunits (6). In addition, the palm subdomain of these DNA polymerases is similar to that of family X members, suggesting that pol III family is more closely related to PolXs than to eukaryotic and archaeal replicative DNA polymerases (7, 8). Although a proofreading activity for the PHP domain of *Thermus thermophilus* pol III α-subunit has been suggested (9), the polymerization errors introduced by these DNA polymerases are corrected in vivo by the transacting exonuclease activity provided by the ϵ-subunit (10). The structural resolution of *Thermus aquaticus* and *Escherichia coli* pol III α-subunit shows the PHP and polymerization active sites 35-Å apart, as are the editing and polymerase sites in pol I and pol II DNA polymerases (7, 8). However, in these cases, the two active sites lie on opposite faces of the enzyme, requiring substantial structural rearrangements that allow proofreading of the polymerization errors by the PHP domain. Thus, it is tempting to speculate on an additional role for pol III members in DNA repair processes, maybe in the recognition and incision of abasic sites.

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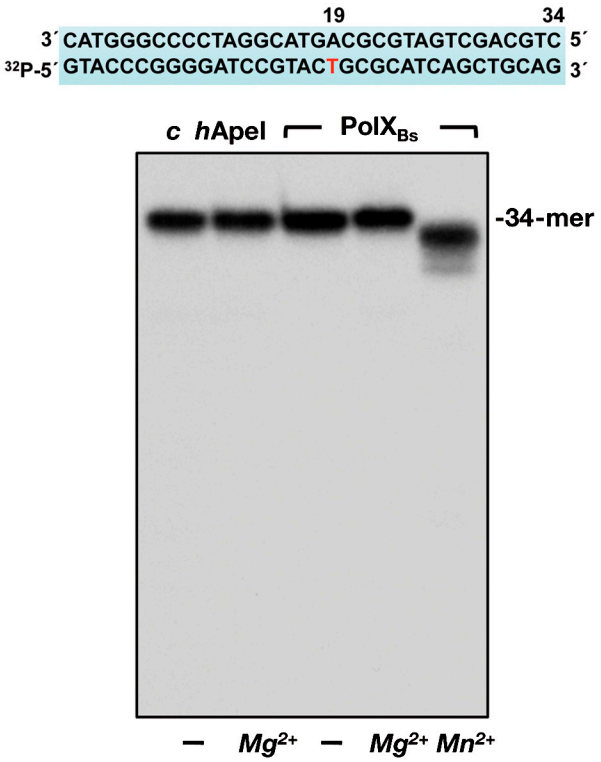

Fig. S1. $PolX_{Bs}$ does not cleave at internal positions of a nondamaged dsDNA. The assay was performed as described in *Materials and Methods* incubating 1.5 nM of the dsDNA depicted on top of figure, 125 nM of $PolX_{Bs}$ and, when indicated, either 8-mM $MgCl_2$ or 1-mM $MnCl_2$ for 10 min at 30 °C.

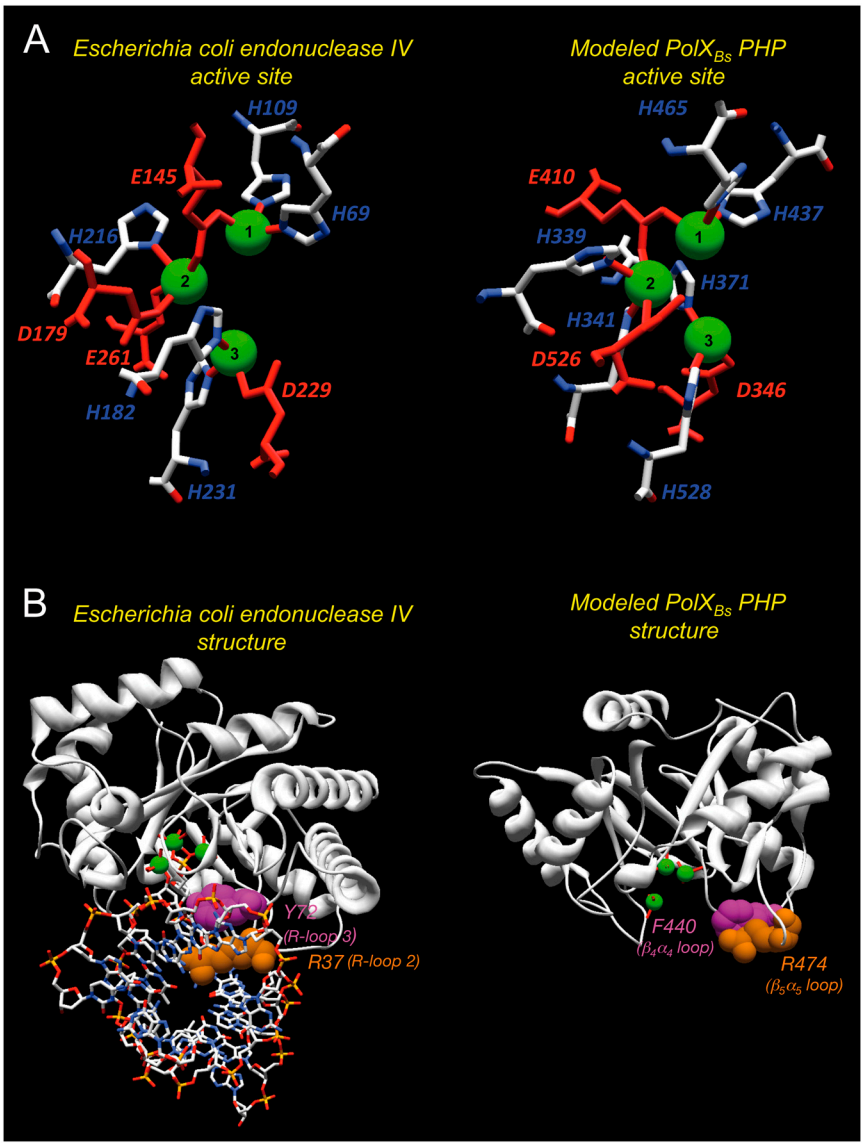

Fig. S2. Structural convergence between the catalytic sites of *E. coli* EndoIV and PolX$_{Bs}$ PHP domain. (*A*) The EndoIV active site crystallographic data are from Protein Data Bank (PDB) 1QTW. PolX$_{Bs}$ PHP domain model was provided by the homology-modeling server Swiss-Model (11, 12), using as template the crystallographic coordinates of *Deinococcus radiodurans* PolX (PDB 2W9M; ref. 3). Both active sites were similarly oriented to emphasize their structure/function similarities. (*B*) *E. coli* EndoIV and modeled PHP domain of PolX$_{Bs}$. EndoIV residues Y72 (R-loop 3) and R37 (R-loop 2) intercalate in the DNA, stacking with the base pair 3′ and 5′ to the abasic site, respectively. The proposed homolog residues F440 (β_4-α_4 loop) and R474 (β_5-α_5 loop) of PolX$_{Bs}$ PHP domain are represented in a similar orientation.

Table S1. Enzymatic activities of wild-type and mutant derivatives of PolX$_{Bs}$

	PolX$_{Bs}$*							
Activity assayed	Wild type	D346A	H371A	E410A	H437A	H465A	D526A	H528A
3′–5′ exonuclease	100	2.8	0.6	ND	ND	ND	ND	0.5
AP endonuclease	100	7	2	0.6	3	ND	ND	8
Polymerization†	100	75	80	67	92	48	45	50

Polymerization activity of mutant derivatives respect to that of the wild-type enzyme (%) was calculated from the Cerenkov radiation of the exclude volume. ND, not detected under the experimental conditions used.
*Numbers indicate the percentage of activity relative to the wild-type enzyme, obtained from several experiments.
†DNA polymerization activity assays on activated DNA. The assay was performed essentially as described in ref. 4.