DNA Synthesis and dRPase Activities of Polymerase β Are Both Essential for Single-Nucleotide Patch Base Excision Repair in Mammalian Cell Extracts

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ABSTRACT: In mammalian cells the majority of altered bases in DNA are processed through a single-nucleotide patch base excision repair mechanism. Base excision repair is initiated by a DNA glycosylase that removes a damaged base and generates an abasic site (AP site). This AP site is further processed by an AP endonuclease activity that incises the phosphodiester bond adjacent to the AP site and generates a strand break containing 3′-OH and 5′-sugar phosphate ends. In mammalian cells, the 5′-sugar phosphate is removed by the AP lyase activity of DNA polymerase β (Pol β). The same enzyme also fills the gap, and the DNA ends are finally rejoined by DNA ligase. We measured repair of oligonucleotide substrates containing a single AP site in cell extracts prepared from normal and Pol β-null mouse cells and show that the reduced repair in Pol β-null extracts can be complemented by addition of purified Pol β. Using this complementation assay, we demonstrate that mutated Pol β without dRPase activity is able to stimulate long patch BER. Mutant Pol β deficient in DNA synthesis, but with normal dRPase activity, does not stimulate repair in Pol β-null cells. However, under conditions where we measure base excision repair accomplished exclusively through a single-nucleotide patch BER, neither dRPase nor DNA synthesis mutants of Pol β alone, or the two together, were able to complement the repair defect. These data suggest that the dRPase and DNA synthesis activities of Pol β are coupled and that both of these Pol β functions are essential during short patch BER and cannot be efficiently substituted by other cellular enzymes.

Base excision repair (BER)[1] operating in human cells provides an efficient mechanism for protection against accumulation of altered bases in DNA. BER is initiated by a DNA glycosylase, which removes the modified base, and then an apurinic/apyrimidinic endonuclease (AP endonuclease) binds to the AP site and hydrolyzes the phosphodiester bond 5′ to the abasic site (*1*). This single-strand DNA break cannot be repaired directly by DNA ligase because it contains a 5′-terminal deoxyribose phosphate (dRP); thus, dRP must be removed to allow further repair. The enzymes that are able to remove the 5′-end sugar phosphate are collectively named DNA deoxyribophosphodiesterases (dRPases), and can be divided into two classes: AP lyases and hydrolytic dRPases (*2*−*4*). The hydrolytic dRPases have been partially purified from bacterial and human cells. These dRPases are Mg^{2+}-dependent and release chemically unaltered sugar phosphate (*5*, *6*). In contrast, AP lyases remove dRP residues through a β-elimination reaction and release sugar as a 3′-α, β-unsaturated aldehyde (*7*, *8*). The fapy-DNA glycosylase (FPG) protein is the major AP lyase in bacterial cells (*9*), but in mammalian cells the major AP lyase activity is associated with DNA polymerase β (Pol β) (*10*, *11*). Pol β removes 5′-sugar phosphate and simultaneously adds one nucleotide to the 3′-end of the nick (*10*). The nick is finally sealed by DNA ligase, and the entire repair reaction results in the removal and replacement of a single nucleotide (single-nucleotide patch BER). The single-nucleotide patch BER is the major BER pathway in both bacterial and mammalian cells (*12*−*14*) and is coordinated by multiple protein-protein interactions (*1*, *15*−*17*). The key proteins in the single-nucleotide patch BER pathway interact with each other. Human AP endonuclease 1 (APE1) interacts with Pol β (*18*). In turn, Pol β directly interacts with DNA ligase I, or through the XRCC1 protein with DNA ligase III (*19*−*21*). Thus, the system is well tuned, and it is not known whether other cellular proteins can substitute for any of its components. An important question is whether Pol β is the only enzyme with substantial dRPase activity and the only DNA polymerase that is able to fill a single-nucleotide gap in the coordinated reaction of single-nucleotide patch BER in mammalian cells. Also, how much are the BER components interchangeable with other cellular enzymes, and what backup systems exist if the major components are knocked out by mutation or underexpressed? The Pol β-null mouse cells provide a unique model to address these questions. We used an in vitro DNA repair complementation assay to exclusively detect single-nucleotide patch BER and demonstrate that both dRPase and DNA synthesis functions of Pol β are essential for its role in short patch BER and that neither

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[1] Abbreviations: BER, base excision repair; AP sites, apurinic/apyrimidinic sites, abasic sites; APE1, apurinic/apyrimidinic endonuclease 1; PCNA, proliferating cell nuclear antigen; FEN1, flap endonuclease; Pol β, DNA polymerase β; dRP, 5′-deoxyribose phosphate; UDG, uracil-DNA glycosylase.

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of these functions can be efficiently substituted by other cellular enzymes.

MATERIALS AND METHODS

Materials. Synthetic oligodeoxyribonucleotides purified by high-pressure liquid chromatography were obtained from Midland. [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham Pharmacia Biotech. Recombinant human Pol β, Pol β K72A, Pol β R283A, and uracil-DNA glycosylase (UDG) were purified as described (*22*, *23*). DNA ligase I was a gift from A. Tomkinson. Histidine-tagged human APE1 and FEN1 proteins were purified on Ni^{2+}-charged His-Bind Resin (Novagen, Cambridge, MA) as recommended by the manufacturer. Whole cell extracts were prepared by the method of Manley et al. (*24*) from DNA Pol β-knockout mouse fibroblasts (MB19tsA) and the isogenic wild-type cells (MB16tsA). Cells were grown as described (*25*).

Reconstituted BER Reaction Using an Oligonucleotide DNA Substrate. The 33-mer oligonucleotide containing a single uracil residue at position 17 was 5′-end-labeled and annealed to the complementary strand as previously described (*26*). Prior to assembly of the excision reaction, the oligonucleotide substrate (100 ng) was pretreated with UDG (200 ng) in 10 mM Hepes, pH 7.9, 1 mM EDTA, and 70 mM KCl. The reaction mixture was incubated at 37 °C for 30 min. Due to the instability of the AP-containing DNA, the substrates were prepared just before performing the BER reactions. The BER reaction was reconstituted in a reaction mixture (10 μL) that contained 45 mM Hepes, pH 7.8, 70 mM KCl, 2 mM DTT, 7.5 mM $MgCl_2$, 0.5 mM EDTA, 2 mM ATP, 20 μM each of the indicated dNTPs, and ^{32}P-labeled oligonucleotide substrate (10 ng). The reactions were initiated by addition of whole cell extracts (1−2 μg) and/or purified APE1, FEN1, and Pol β, at the amount indicated in the figure legends. After incubation for 20 min at 37 °C, the reactions were stopped by addition of 10 μL of gel loading buffer (95% formamide, 20 mM EDTA, 0.02% bromophenol blue, and 0.02% xylene cyanol). Following incubation at 90 °C for 2−5 min, the reaction products were separated by electrophoresis in a 20% polyacrylamide gel containing 8 M urea, 89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA, pH 8.0. All experiments were repeated at least 3−5 times, and representative gels are shown.

RESULTS

Characterization of in Vitro BER. To monitor BER supported by whole cell extracts (WCE), we used oligonucleotide duplex substrates containing a single uracil/adenine base pair. The substrates were 5′-end-labeled in the uracil-containing strand (Figure 1A). Prior to the reactions, uracil was removed by incubation with human uracil-DNA glycosylase (UDG) to generate an abasic site. Incubation of the AP-containing substrate with whole cell extract in the absence of deoxyribonucleotide triphosphates (dNTPs) resulted in the incision of the oligonucleotides at the AP site, generating a 16-mer incision product (Figure 1B, lane 2). Due to the lack of NTPs, further repair was blocked, and no full-length product was generated. Instead, the incised substrate was subjected to limited degradation by the cell extract (Figure 1B, lane 2). However, when dNTPs were added, the repair was completely accomplished within 20

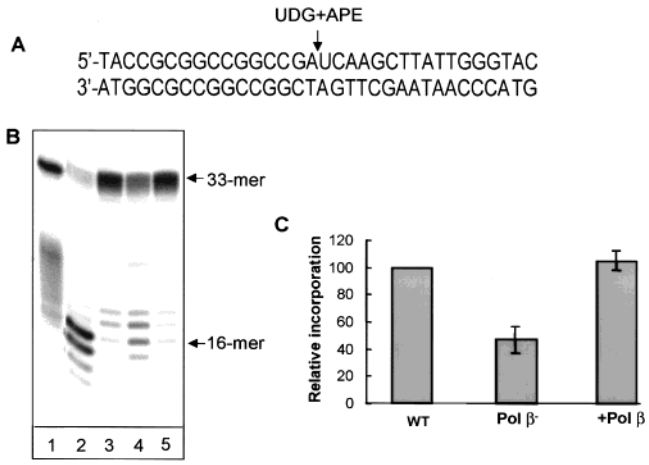

FIGURE 1: Complementation of BER deficiency in Pol β-null cell extracts by purified Pol β. (A) Uracil-containing oligonucleotide substrate. The arrow indicates the incision site generated by the combined action of UDG and APE. (B) A 5′-end-labeled 33 bp duplex oligonucleotide (10 ng) containing a uracil residue at position 17 was pretreated with UDG to generate an AP site and then was incubated with WCE (10 μg) as described under Materials and Methods. Reactions were carried out at 37 °C for 20 min and stopped by addition of an equal volume of formamide−dye solution, and products were analyzed by electrophoresis in a 20% denaturing polyacrylamide gel. Lane 1, no extract added; lane 2, Pol β-null extract, no dNTPS added; lane 3, complete reaction with wild-type extract; lane 4, complete reaction with Pol β-null extract; lane 5, Pol β-null extract complemented with 2 ng of wild-type Pol β. (C) PhosphoroImager quantification of lanes 3−5 from panel B is shown. The results were quantified with PhosphoroImager and normalized to the incorporation in the wild-type cell extract.

min, and most of the incision products were converted to the full-length 33-mer product (Figure 1B, lane 3). As previously reported (*25*), this reaction was dependent on the Pol β status of the cell extracts, and we found that after 20 min of incubation the repair activity in Pol β-null cell extracts was 2.3-fold lower than in normal cell extracts (Figure 1B, compare lanes 3 and 4, and Figure 1C). This repair activity of Pol β-null cell extracts can be complemented to the level of normal cell extracts by addition of purified Pol β (Figure 1B, lane 5, and Figure 1C). These data indicate that under our reaction conditions the decrease in repair activity in Pol β-null cell extracts is mainly due to the absence of Pol β and that all other BER components are not limiting.

Complementation of BER in Pol β-null Cell Extracts by Pol β Deficient in dRPase or DNA Polymerase Activity. We then addressed the question of whether dRPase and DNA synthesis mutants of Pol β could complement Pol β-null cell extracts. The crystal structure of Pol β has been solved (*27*), and proteins deficient in DNA synthesis or dRPase activity have been generated and biochemically characterized (*28*). It has been shown that substitution of Lys72 for alanine (Pol β K72A) in the dRPase active site nearly eliminates dRPase function, but does not affect DNA synthesis activity (*29*). Similarly, substitution of Arg283 for alanine (R283A) selectively affects only DNA synthesis activity (*30*). We used a BER complementation assay to characterize the ability of these mutated proteins to restore BER in Pol β-null cell extracts. In the first series of experiments, we have used a 33-mer 5′-labeled substrate containing an AP site (Figure 1A) and normal dNTPs. Both wild-type and the dRPase (K72A) mutants, but not the DNA synthesis (R283A) mutant

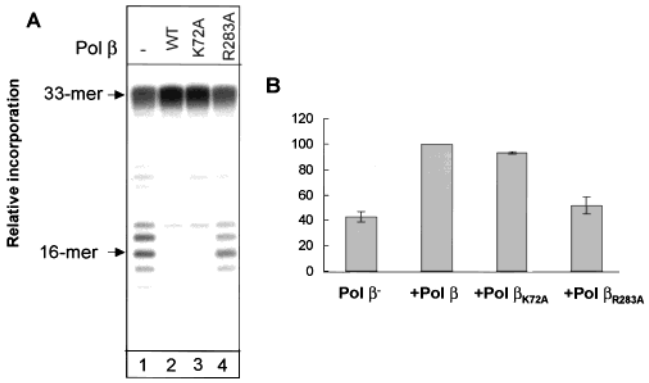

FIGURE 2: dRPase-deficient Pol β stimulates long patch BER in Pol β-null cell extracts. (A) The reaction conditions and product analyses are described under Materials and Methods. Reaction mixtures containing 5′-end-labeled 33 bp duplex oligonucleotide substrate (Figure 1A, 10 ng) pretreated with UDG were incubated with Pol β-null cell extract (10 μg) complemented with 2 ng of the indicated Pol β protein. Reactions were incubated for 20 min at 37 °C and stopped by addition of an equal volume of formamide−dye solution, and products were analyzed by electrophoresis in a 20% denaturing polyacrylamide gel. (B) PhosphoroImager quantification of the results presented in panel A is shown. The results were quantified with PhosphoroImager and normalized to the incorporation stimulated by wild-type Pol β.

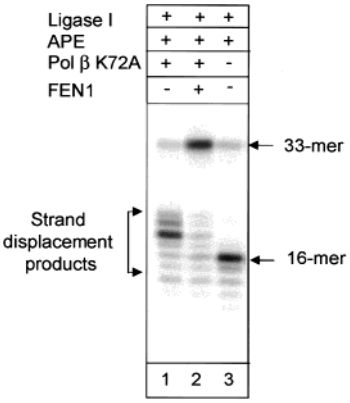

FIGURE 3: Reconstitution of long patch BER with Pol β K72A. A 33 bp duplex oligonucleotide (10 ng) containing a uracil residue at position 17 was pretreated with UDG to generate an AP site. The buffer containing magnesium, dNTPs, and the indicated proteins [10 ng of APE1, 2 ng of dRPase-deficient mutant of Pol β (Pol βK72A), and 10 ng of FEN1] was then added, and incubation was carried out for 20 min at 37 °C. Reactions were stopped by addition of an equal volume of formamide−dye solution, and products were analyzed by electrophoresis in a 20% denaturing polyacrylamide gel.

of Pol β, were able to stimulate repair incorporation in the Pol β-null extracts (Figure 2A, B). Pol β K72A does not have dRPase activity; thus, it should not be able to stimulate single nucleotide patch repair unless it is cooperating with some other cellular dRPases. Alternatively, this mutant protein may influence long patch repair by stimulating strand displacement. Indeed, in repair reactions reconstituted with purified APE and DNA ligase, APE incised the AP site and generated a 16-mer product, but DNA ligase was not able to ligate it (Figure 3, lane 3). Pol β K72A mutant protein, when added, was not able to support single-nucleotide patch BER, but efficiently stimulated strand displacement synthesis (Figure 3, lane 1). Repair was completely accomplished when the Pol β K72A containing reaction was supplemented with FEN1 (Figure 3, lane 2). FEN1 is not able to remove dRP

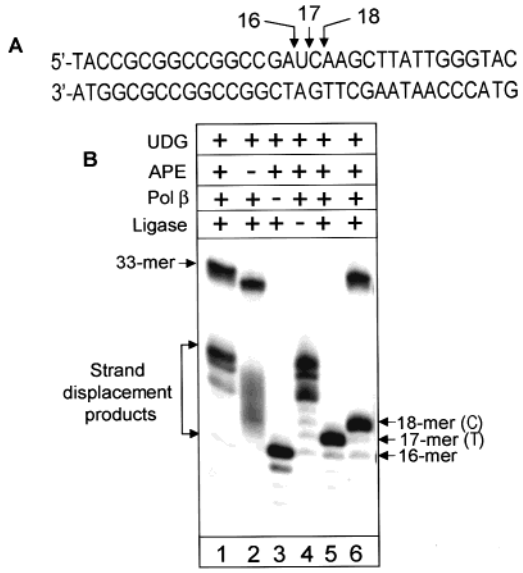

FIGURE 4: Single-nucleotide BER reconstituted with purified proteins. The reaction conditions and product analyses are described under Materials and Methods. 10 ng of a 33 bp duplex oligonucleotide (A) containing a uracil residue at position 17 was pretreated with uracil−DNA glycosylase to generate an AP site. (B) The buffer containing magnesium, dNTPs, and the indicated proteins (10 ng of APE1, 2 ng of Pol β, and 35 ng of DNA ligase I) was then added, and incubation was carried out for 20 min at 37 °C. Normal dNTPs were used in the reactions shown in lanes 1−4. The reaction shown in lane 5 contained all ddNTPs, and the reaction in lane 6 contained dTTP and the rest of the nucleotides were ddNTPs. Reactions were stopped by addition of an equal volume of formamide−dye solution, and products were analyzed by electrophoresis in a 20% denaturing polyacrylamide gel.

residues alone but can release them as part of an oligonucleotide 2−6 nucleotides long (*31*). We thus suggested that the Pol β K72A mutant is able to support long patch repair by stimulating strand displacement.

dRPase and DNA Synthesis Mutants of Pol β Cannot Stimulate Single-Nucleotide Patch BER in Pol β-null Cell Extracts. To address the question whether K72A or R283A proteins are able to stimulate single-nucleotide patch BER, we used an assay that exclusively measures single-nucleotide patch repair products. This method is based on the ability of dideoxyribonucleotide triphosphates (ddNTPs) to terminate both DNA repair synthesis by DNA polymerase and end-rejoining by DNA ligase. We added a single normal dNTP to the repair reaction (the one that substitutes the damaged base), and the rest were ddNTPs. Under these reaction conditions, incorporation of any second nucleotide into the repair gap should block further repair. Thus, only incorporation of the first normal nucleotide into the repair gap (single-nucleotide patch) would create a substrate suitable for ligation and would generate a repaired product. To demonstrate the efficiency of this approach, we reconstituted single-nucleotide BER with four purified human proteins: UDG, APE1, Pol β, DNA ligase, and a 5′-end labeled uracil-containing oligonucleotide duplex as a substrate (Figure 4A). Each added protein was essential for the reconstituted BER reaction. UDG removes uracil from the oligonucleotide; however, in the absence of APE1, the arising AP site was not processed further (Figure 4B, lane 2). In the absence of Pol β, only a 16-mer incision product was formed (Figure 4B, lane 3), and there were no full-length products in the

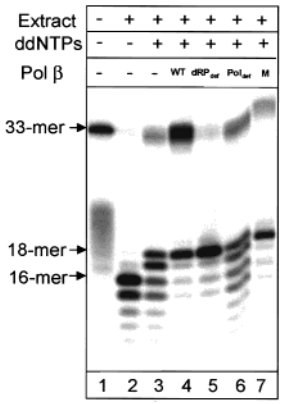

FIGURE 5: Single-nucleotide BER deficiency in Pol β-null cell extracts is not complemented by dRPase or DNA polymerase deficient Pol β. The reaction conditions and product analyses are described under Materials and Methods. Reaction mixtures containing Pol β-null cell extract complemented with 2 ng of the indicated Pol β protein were incubated for 20 min at 37 °C in the presence of dTTP and three ddNTPs. Reactions were stopped by addition of an equal volume of formamide−dye solution, and products were analyzed by electrophoresis in a 20% denaturing polyacrylamide gel. Lane 1, no extract added; lane 2, Pol β-null extract, no dNTPS added; lane 3, complete reaction with Pol β-null extract; lanes 4−7, complete reaction with Pol β-null extract complemented with 2 ng of wild-type Pol β, dRPase-deficient Pol β K72A (72A), DNA synthesis deficient Pol β R283A (283A), and the mixture (M) of Pol β K72A and Pol β R283A, respectively.

absence of DNA ligase (Figure 4B, lane 4). When all four proteins were added in the presence of dNTPs, full-length 33-mer products were observed in addition to strand displacement products (Figure 4B, lane 1). However, when all four dNTPs were substituted with ddNTPs, DNA repair synthesis was terminated after addition of the first nucleotide (17-mer product), and no full-length products emerged (Figure 4B, lane 5). Finally, the combination of dTTP and three other ddNTPs generates full-length repaired products but blocks strand displacement after addition of ddCMP (Figure 4B, lane 6, 18-mer termination product). We thus conclude that these reaction conditions allow DNA repair to proceed via single-nucleotide patch, but strongly blocks the reaction after addition of the second nucleotide into the repair gap.

Using this assay that monitors solely single-nucleotide patch BER, we tested whether either of the Pol β mutants would be able to stimulate this reaction in Pol β-null cell extracts. We incubated 5′-end-labeled substrate in Pol β-null cell extracts and in Pol β-null cell extracts complemented with wild-type or mutant Pol β (K72A or R283A) proteins (Figure 5 , lanes 1−5). We found that only wild-type Pol β, but none of the mutated proteins alone or in combination, was able to stimulate single-nucleotide patch repair. We thus conclude that both activities of Pol β are required simultaneously during short patch BER and other enzymes present in a whole cell extract cannot complement these activities.

DISCUSSION

Pol β is the major DNA polymerase participating in the single-nucleotide patch pathway that normally performs the majority of BER (*11, 25*). The Pol β-deficient mice are not viable (*32*), but corresponding Pol β-null embryonic cells survive in cell culture (*25*). These cells have normal growth characteristics, but are hypersensitive to alkylating agents and have increased rates of chromosomal abnormalities (*33*). The viability of the Pol β-null cells indicates that some other DNA repair pathways exist in these cells. Indeed, a Pol β-independent single-nucleotide patch pathway for repair of 8-oxoguanine was previously observed in mammalian cell extracts (*34, 35*). Nevertheless, because the knockout mice are not viable, it is likely that the efficiency or quality of this repair initiated by DNA glycosylases with putative AP-lyase activity is insufficient to maintain the genome integrity during development of a multicellular organism. Indeed, in accordance with published results (*25*), we found a 2−3-fold decrease in the ability to repair AP sites in Pol β-null cell extracts. This repair defect can be complemented by wild-type Pol β, but, surprisingly, none of the mutant proteins studied here, alone or in combination, were able to stimulate single-nucleotide patch BER. Pol β K72A mutant protein binds to the substrate containing a dRP residue (*29*), but possibly because it is unable to make a stable intermediate with dRP or to remove it, this protein stimulates uncontrolled strand displacement and shifts the repair process to the FEN1-dependent long patch BER. On the other hand, Pol β R283A can remove the dRP residue and generate a one nucleotide gap that may be filled by other polymerases present in the cell extract. Thus, inability of Pol β R283A to stimulate single-nucleotide patch BER in Pol β-null cell extracts indicates that a one nucleotide gap repair intermediate is not a good substrate for other cellular polymerases. It is likely that 5′-dRP residues at the preincised AP site have dual roles: they attract Pol β and at the same time limit the strand displacement to one nucleotide only. This may occur because Pol β loses affinity for the substrate and dissociates from the complex after removal of the dRP. Indeed, previous studies have shown that the presence of a 5′-phosphate is crucial for Pol β binding (*36*). This model also explains why a combination of two enzymes does not stimulate single-nucleotide BER. If Pol β K72A first binds the substrate, it will shift repair to the long patch pathway. On the other hand, if Pol β R 283A binds first, it removes the dRP residue, and the resulting one nucleotide gap is not a good substrate for Pol β K72A to bind. To test this model, some additional experiments using gel retardation technique and order of addition experiments are currently in progress. Taken together, these results suggest that the two functions of Pol β are coordinated and both play an essential role in the coupled BER reactions consisting of the removal of the 5′-sugar phosphate and filling the one nucleotide gap during repair of abasic sites.

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REFERENCES

1. Lindahl, T., and Wood, R. D. (1999) *Science 286*, 1897−1905.
2. Lindahl, T. (1990) *Mutat. Res. 238*, 305−311.
3. Bailly, V., and Verly, W. G. (1989) *Nucleic Acids Res. 17*, 3617−3618.
4. Doetsch, P. W., and Cunningham, R. P. (1990) *Mutat. Res. 236*, 173−201.
5. Franklin, W. A., and Lindahl, T. (1988) *EMBO J. 7*, 3617−3622.
6. Price, A., and Lindahl, T. (1991) *Biochemistry 30*, 8631−8637.
7. Bailly, V., and Verly, W. G. (1988) *Biochem. J. 253*, 553−559.

8. McCullough, A. K., Dodson, M. L., and Lloyd, R. S. (1999) *Annu. Rev. Biochem. 68*, 255−285.
9. Graves, R. J., Felzenszwalb, I., Laval, J., and O'Connor, T. (1992) *J. Biol. Chem. 267*, 14429−14435.
10. Matsumoto, Y., and Kim, K. (1995) *Science 269*, 699−702.
11. Sobol, R. W., Prasad, R., Evenski, A., Baker, A., Yang, X.-P., Horton, J., and Wilson, S. H. (2000) *Nature 405*, 807−809.
12. Dianov, G., Price, A., and Lindahl, T. (1992) *Mol. Cell. Biol. 12*, 1605−1612.
13. Dianov, G., Bischoff, C., Piotrowski, J., and Bohr, V. A. (1998) *J. Biol. Chem. 273*, 33811−33816.
14. Dianov, G. L., Thybo, T., Dianova, I. I., Lipinski, L. J., and Bohr, V. A. (2000) *J. Biol. Chem. 275*, 11809−11813.
15. Wilson, S. H., and Kunkel, T. A. (2000) *Nat. Struct. Biol. 7*, 176−178.
16. Mol, C. D., Izumi, T., Mitra, S., and Tainer, J. A. (2000) *Nature 403*, 451−454.
17. Rice, P. A. (1999) *Nat. Struct. Biol. 6*, 805−806.
18. Bennett, R. A., Wilson, D. M., III, Wong, D., and Demple, B. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 7166−7169.
19. Prasad, R., Singhal, R. K., Srivastava, D. K., Molina, J. T., Tomkinson, A. E., and Wilson, S. H. (1996) *J. Biol. Chem. 271*, 16000−16007.
20. Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D., and Lindahl, T. (1996) *EMBO J. 15*, 6662−6670.
21. Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. (1996) *Nucleic Acids Res. 24*, 4387−4394.
22. Kumar, A., Widen, S., Williams, K., Kedar, P., Karpel, R., and Wilson, S. (1990) *J. Biol. Chem. 265*, 2124−2131.
23. Slupphaug, G., Eftedal, I., Kavli, B., Bharati, S., Helle, N. M., Haug, T., Levine, D. W., and Krokan, H. E. (1995) *Biochemistry 34*, 128−138.
24. Manley, J. L., Fire, A., Samuels, M., and Sharp, P. (1983) *Methods Enzymol. 101*, 568−582.
25. Sobol, R. W., Horton, J. K., Kuhn, R., Gu, H., Singhal, R. K., Prasad, R., Rajewsky, K., and Wilson, S. H. (1996) *Nature 379*, 183−186.
26. Dianov, G., and Lindahl, T. (1991) *Nucleic Acids Res. 19*, 3829−3833.
27. Pelletier, H., Sawaya, M. R., Wolfle, W., Wilson, S. H., and Kraut, J. (1996) *Biochemistry 35*, 12742−12761.
28. Wilson, S. H. (1998) *Mutat. Res. 407*, 203−215.
29. Prasad, R., Beard, W. A., Chyan, J. Y., Maciejewski, M. W., Mullen, G. P., and Wilson, S. H. (1998) *J. Biol. Chem. 273*, 11121−11126.
30. Beard, W. A., Osheroff, W. P., Prasad, R., Sawaya, M. R., Jaju, M., Wood, T. G., Kraut, J., Kunkel, T. A., and Wilson, S. H. (1996) *J Biol. Chem. 271*, 12141−12144.
31. DeMott, M. S., Shen, B., Park, M., Bambara, R. A., and Zingman, S. (1996) *J. Biol. Chem. 47*, 30068−30076.
32. Gu, H., Marth, J. D., Orban, P. C., Mossman, H., and Rajewsky, K. (1994) *Science 265*, 103−106.
33. Ochs, K., Sobol, R. W., Wilson, S. H., and Kaina, B. (1999) *Cancer Res. 59*, 1544−1551.
34. Dianov, G., Bischoff, C., Sunesen, M., and Bohr, V. A. (1999) *Nucleic Acids Res. 27*, 1365−1368.
35. Fortini, P., Parlanti, E., Sidorkina, O. M., Laval, J., and Dogliotti, E. (1999) *J. Biol. Chem. 274*, 15230−15236.
36. Prasad, R., Beard, W. A., and Wilson, S. H. (1994) *J. Biol. Chem. 269*, 18096−18101.

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