Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 300 (2003) 182–187
BBRC
www.elsevier.com/locate/ybbrc

AP endonuclease 1 has no biologically significant 3′→5′-exonuclease activity☆

Natalia A. Lebedeva,[a,b] Svetlana N. Khodyreva,[a] Alain Favre,[b] and Olga I. Lavrik[a,b,*]

[a] *Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Prospect Lavrentiev 8, 630090, Novosibirsk, Siberia, Russia*
[b] *Institut Jacques Monod (CNRS, Universite Paris 6, Universite Paris 7), 75351 Paris cedex 05, France*

Received 5 November 2002

Abstract

The 3′ → 5′-exonucleolytic activity of human apurinic/apyrimidinic endonuclease 1 (APE1) on mispaired DNA at the 3′-termini of recessed, nicked or gapped DNA molecules was analyzed and compared with the primary endonucleolytic activity. We found that under reaction conditions optimal for AP endonuclease activity the 3′ → 5′-exonuclease activity of APE1 manifests only at enzyme concentration elevated by 6–7 orders of magnitude. This activity does not show a preference to mismatched compared to matched DNA structures as well as to nicked or gapped DNA substrates in comparison to recessed ones. Therefore, the 3′ → 5′-exonuclease activity associated with APE1 can hardly be considered as key mechanism that improves fidelity of DNA repair.

Keywords: Apurinic/apyrimidinic endonuclease; Base excision repair; 3′ → 5′-exonuclease activity

Base excision repair is one of the main strategies used by the cell to defend against exogenous or endogenous genotoxic stress, which otherwise leads to single-base lesions in DNA [1,2]. The currently accepted model for mammalian BER involves two pathways [3–6]. In each of the BER sub-pathways repair can be initiated by spontaneous base loss or DNA glycosylase action to produce an abasic site. When the intact abasic site is an intermediate, DNA strand cleavage on the 5′-side of sugar is catalyzed by apurinic/apyrimidinic endonuclease 1 (APE1), whereas cleavage on the 3′-side of the sugar is performed by the deoxyribose phosphate (dRP) lyase activity of DNA polymerase β (β-pol) [7,8]. The single-nucleotide gap is filled up by β-pol and then the nick is eventually sealed by a DNA ligase thus completing the short-patch or single-nucleotide BER sub-pathway [4,9,10]. In mammalian cells repair of methylated bases, oxidized bases, and abasic sites appears to occur predominantly by this pathway [11–13]. In other cases, for example, when the sugar of the abasic site is not removed efficiently, the long-patch BER pathway mediates repair [14,15]. APE1 is an abundant nuclear enzyme that is responsible for the AP-site repair and found associated with a 3′ → 5′-exonuclease activity. This activity however, was considered to be biologically insignificant because it is 2–4 orders of magnitude lower than AP endonuclease activity [16,17]. It was shown recently that APE1 is able to remove anticancer nucleoside analogs having the stereo chemically unnatural L-configuration [18]. The effect of base–base hydrogen bonding on 3′ → 5′-exonuclease activity of APE1 was also analyzed and it was concluded that APE1 removes 3′-mismatched nucleotides at least 50 times more efficiently than those matched correctly [19]. The latter activity was inversely proportional to the gap size in DNA. Therefore, APE1 can be a major contributor to the fidelity of base excision repair in the short BER pathway. This looks very important since DNA polymerase β lacks a proofreading 3′ → 5′-exonuclease activity and is prone to errors, misincorporating

☆ *Abbreviations:* BER, base excision repair; APE1, apurinic/apyrimidinic endonuclease; β-pol, DNA polymerase β; F, tetrahydrofuran; PAGE, polyacrylamide gel electrophoresis.

* Corresponding author. Fax: +7-3832-33-36-77.
E-mail address: lavrik@niboch.nsc.ru (O.I. Lavrik).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02808-5

1 nucleotide in 4000 [8]. It was shown that DNA polymerase β is cooperative together with APE1 in BER [20]. Therefore, it can be suggested that APE1 does not leave the abasic site immediately after DNA cleavage and can make error corrections after the filling of the gap by DNA polymerase β prior to the cleavage of the sugar phosphate by the lyase activity of β-pol. If this mechanism is operative, the presence of sugar phosphate moiety at the 5′-margin of nick can stimulate removal of mismatched terminal nucleotide. To examine this possibility we used nicked, gapped or recessed DNA structures containing mismatched and matched 3′-termini. Nicked and gapped DNA structures contained phosphate or sugar phosphate (tetrahydrofuranophosphate) at 5′-terminus of downstream primers. For the three different preparations of APE1 obtained independently we did not find efficient 3′ → 5′-exonuclease activity of APE1 with respect to all DNAs used. Under conditions optimal for AP endonuclease activity the 3′ → 5′-exonuclease activity was found to be 6–7 orders of magnitude lower than the endonuclease activity. We did not find a remarkable stimulation of exonuclease activity for mismatched over the matched structures or evident preference of nicked or gapped DNAs in comparison to recessed DNA structure. Therefore, we conclude that this activity of APE1 is possibly the consequence of either minor traces of contaminating exonuclease or is due to removal of the 3′-terminal moiety with low efficiency by the endonucleolytic active site of this enzyme.

Materials and methods

[γ-^{32}P]ATP was from Amersham Pharmacia Biotech, NJ. Synthetic oligonucleotides were obtained from GENSET (France). Reagents for electrophoresis and basic components of buffers were from Sigma (USA).

Protein purification. Human APE1 was purified from of *Escherichia coli* strain BL21/DE3pLysS carrying pXC53 containing human APE1 gene. The plasmid was a gift of Dr. Samuel H. Wilson (NIEHS, NC). Cells from a 2-L culture (6 g) were lysed in Buffer A (50 mM Tris–HCl, pH 8.0, 2% Nonidet P-40, 0.4 M NaCl, 2 mM EDTA, and 7 mM βME) by means of sonic disruption at 0–1 °C. After removal of cell debris by centrifugation at 15,000 rpm for 35 min (centrifuge "Centrikon T-42K," Kontron Instruments, France), the supernatant fraction was adjusted to 0.15 M NaCl by addition of Buffer B (50 mM Tris–HCl, pH 8.0, 0.1% Nonidet P-40, 1 mM EDTA, and 10 mM βME) and mixed with 20 ml DEAE-toyopearl (Toyosoda, Japan), equilibrated with Buffer B + 0.1 M NaCl. The resin was removed by filtration through glass filter and supernatant was loaded on heparin–Sepharose (Pharmacia) column (12 ml) followed by washing with Buffer C (50 mM Tris–HCl, pH 8.0, 1 mM EDTA, 7 mM βME). Bound proteins were eluted with linear gradient of NaCl (0.1–0.6 M) in Buffer C. APE1, eluting at 0.35 M NaCl, was further purified by chromatography on a BioRex 70 (Bio-Rad) column. Gradient was 0–0.5 M NaCl with Buffer C. APE1, eluting at 0.35 M NaCl, after dialysis was loaded on SP-toyopearl column. APE1 eluted from this column at 0.2 M NaCl. Dialysis was carried out overnight against Buffer C and then against Buffer D (50% glycerol, 50 mM Tris–HCl, pH 8.0, 1 mM EDTA, and 5 mM DTT). Preparation of APE1 was homogeneous according to the data of PAGE.

Recombinant rat DNA polymerase β (β-pol) was purified according to [21]. Plasmid pRSET, containing the rat DNA polymerase β gene, was a gift of Dr. Samuel H. Wilson (NIEHS, NC). Other APE1 preparations used in experiments were purified according to [22] and were gifts of Dr. Rajendra Prasad (NIEHS, NC) and Dr. Murat Saparbaev (Institute Gustave Roussy, Villijuif, France).

Radioactive labeling of oligonucleotide primers. Oligodeoxynucleotides were 5′-^{32}P-phosphorylated with T4 polynucleotide kinase (New England Biolabs) as described [23]. Unreacted [γ^{32}P]ATP was separated by passing the mixture over a MicroSpin G-25 column (Amersham Pharmacia Biotech, NJ) using the manufacturer's suggested protocol.

Primer–template annealing. Lyophilized oligonucleotides were dissolved in 10 mM Tris–HCl, pH 7.8, and 1 mM EDTA. Complementary oligodeoxynucleotides were annealed by heating a solution of equimolar amounts to 90 °C for 3 min, followed by slow cooling to room temperature. The sequences of the oligonucleotides used in the experiments were as follows:

ON1 5′-CTG CAG CTG ATG CGC C-3′
ON2 5′-CTG CAG CTG ATG CGC T-3′
ON3 5′-CTG CAG CTG ATG CGC A-3′
ON4 5′-GTA CCC GGG GAT CCG TAC GGC GCA TCA GCT GCA G-3′
ON5 5′-**p**GT ACG GAT CCC CGG GTA C-3′
ON6 5′-**pF**GT ACG GAT CCC CGG GTA C-3′ (**F** denotes 3-hydroxy-2-hydroxymethyltetrahydrofuran)
ON7 5′-CTG CAG CTG ATG CGC-3′
ON8 5′-CTG CAG CTG ATG CGT-3′
ON9 5′-CTG CAG CTG ATG CGA-3′.

The oligonucleotides were mixed in the following combinations:

	Match C–G	Mismatch T–G	Mismatch A–G
Recessed DNA	ON1 + ON4; ON7 + ON4	ON2 + ON4; ON8 + ON4	ON3 + ON4; ON9 + ON4
Gapped DNA	ON7 + ON5 + ON4; ON7 + ON6 + ON4	ON8 + ON5 + ON4; ON8 + ON6 + ON4	ON9 + ON5 + ON4; ON9 + ON6 + ON4
Nicked DNA	ON1 + ON5 + ON4; ON1 + ON6 + ON4	ON2 + ON5 + ON4; ON2 + ON6 + ON4	ON3 + ON5 + ON4; ON3 + ON6 + ON4

Thirty-four base pair oligonucleotide duplex containing a synthetic abasic site (tetrahydrofuran residue called F) opposite G or opposite A was used for endonucleolytic cleavage of APE1:

5′-[^{32}P]-CTG CAG CTG ATG CGC **F** GT ACG GAT CCC CGG GTA C-3′
3′-GAC GTC GAC TAC GCGG(A)CA TGC CTA GGG GCC CAT G-5′

Exonuclease/endonuclease assays. Standard reaction mixtures (10 μl) contain 50 mM Tris–HCl, pH 8.6, 30 mM KCl, 5 mM $MgCl_2$, and 10 nM 5′-^{32}P-labeled DNA. To initiate cleavage of DNA, the mixture was incubated with different concentrations of APE1 (from 0.1 nM to 1 μM for exonuclease assays and from 100 fM to 1 nM for endonuclease assays) for 30 min at 25 or 37 °C in a standard reaction mixture. The reactions were terminated by adding loading solution (90% formamide, 50 mM EDTA, 0.1% xylene cyanole, and 0.1% bromophenol blue). The mixtures were heated for 3 min at 80 °C and products were analyzed by electrophoresis in 15% polyacrylamide gel with 7 M urea followed by autoradiography [23].

DNA synthesis by β-pol. Standard reaction mixtures (10 μl) contain 50 mM Tris–HCl, pH 8.6, 50 mM KCl, 5 mM $MgCl_2$, 0.2 μM ^{32}P-labeled recessed DNA, and 0.5 μM β-pol. After complete primer elongation by β-pol in the presence of 10 μM TTP (30 min, 37 °C), different concentrations of APE1 were added to the reaction mixture. The reaction was terminated by adding 10 μl of 90% formamide, 50 mM EDTA, 0.1% xylene cyanole, and 0.1% bromophenol blue. The mixtures were heated for 3 min at 80 °C and products were analyzed by electrophoresis followed by autoradiography [23].

Results

Along with the recent data indicating the role of APE1 in the fidelity of DNA repair [19], we decided to analyze the influence of the structure of the 5′-margin of nicked DNA on the 3′ → 5′-exonuclease activity of this enzyme. Indeed, branch-point intermediate of base excision repair contains a sugar phosphate moiety at 5′-margin of nicked DNA structure after endonucleolytic cleavage of DNA nearby apurinic/apyrimidinic site. The nick containing tetrahydrofuranophosphate at 5′-terminus of downstream primer mimics this intermediate. Therefore, this DNA structure can be regarded as a model of branch-point intermediate in BER. Recessed DNA or gapped and nicked DNA structures containing phosphate or sugar phosphate at 5′-terminus of their downstream primers were subjected to exonuclease cleavage at different concentrations of APE1. DNA structures used in the experiments contained either matched (CG) or mismatched base pairs (TG or AG) at 3′-terminus of upstream primers. To examine the 3′ → 5′-exonuclease activity of APE1 we started from conditions described in [19]. However, we found only traces of exonuclease activity using recessed DNA with TG mismatched at the 3′-terminus. This activity was detected at APE1 concentration equal to or higher than 10^{-5} M (Fig. 1, lanes 1–6). The exonuclease activity is inhibited upon increasing the ionic strength (Fig. 1, lanes 7–11) but is stimulated at higher pH (Fig. 1, lanes 12–16). We have made experiments using temperature 37 °C to compare with 25 °C. The

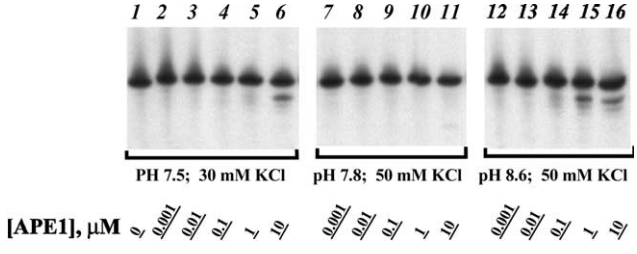

Fig. 1. Dependence of APE1 3′ → 5′-exonuclease activity on the enzyme concentration at different pH and ionic strength. Reaction mixtures contained recessed template–primer system with TG mismatch at 3′-terminus. The products were analyzed by PAGE with subsequent autoradio-graphy.

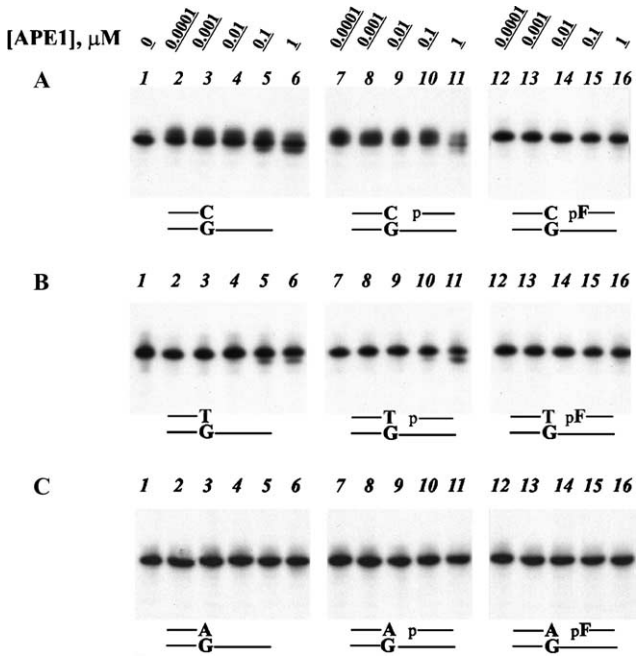

Fig. 2. Dependence of APE1 3′ → 5′-exonuclease activity at different concentrations on DNA structure. Reaction mixtures contained 50 mM Tris–HCl, pH 8.6, 30 mM KCl, 10 nM DNA and defined amount of APE1. The products were separated by denaturing PAGE followed by autoradiography. (A) Represents matched CG structures: recessed DNA (lanes 1–6), one-window gapped DNA carrying phosphate at 5′–margin (lanes 7–11), and one-window gapped DNA carrying tetrahydrofuranophosphate moiety at 5′–margin (lanes 12–16). (B) The same, but with mismatched TG, and (C) with mismatched AG.

regularities are not changed. Based on these data we used pH 8.6 and 30 mM KCl in the following experiments at 25 °C.

The data obtained using recessed and one-window gapped DNA carrying matched and mismatched structures are shown in Fig. 2. The results demonstrate that when the concentration of APE1 varied in a range from 10^{-10} to 10^{-8} M no visible cleavage of DNA (10^{-8} M) was found for both matched and mismatched structures. The efficiency of the nucleotide removal at 3′-terminus is strongly dependent on the enzyme concentration and can be detected only at 10^{-7}–10^{-6} M concentration of APE1. The activity is higher for recessed DNA for both matched (CG) (Fig. 2A) or mismatched (TG) (Fig. 2B) structures than for the respective gapped DNA structures (Figs. 2A and B). The presence of a phosphate at the 5′–terminus of downstream primer in gapped DNA for tetrahydrofuranophosphate moiety completely abolished removal of both matched (CG) and mismatched (TG) nucleotides (compare Figs. 2A and B, lanes 11 and 16). DNAs with AG mismatch were very resistant to 3′ → 5′-exonuclease cleavage. Similar data were obtained for nicked DNA structures (data not shown).

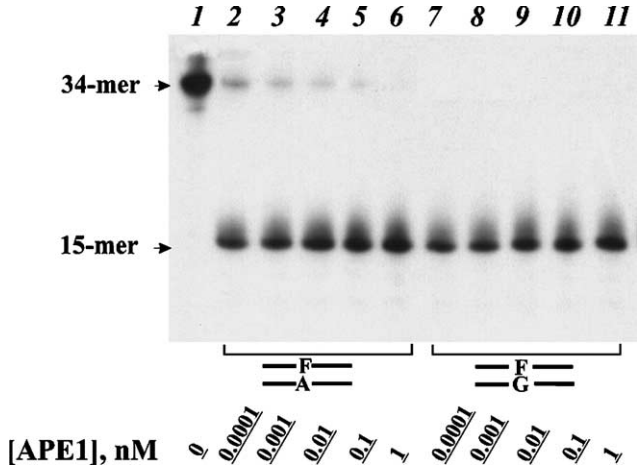

Fig. 3. Specific endonucleolytic cleavage of oligonucleotide containing the synthetic abasic site by APE1. **F** denotes 3-hydroxy-2-hydroxymethyltetrahydrofuran (THF). Defined amounts of APE1 were incubated for 30 min with 10 nM DNA containing the synthetic abasic site on one strand located opposite A (lanes 2–6) or opposite G (lanes 7–11). The products were separated by denaturing PAGE followed by autoradiography.

According to the earlier publications the $3' \rightarrow 5'$-exonuclease activity of APE1 was considered to be insignificant biologically since it is by 2–4 orders of magnitude lower than the AP endonuclease activity [16,17]. However, recent publication is regarding this activity as biologically sound [19]. To address this question we examined the concentration dependence of AP endonuclease activity using DNA with a synthetic abasic site in one strand located either opposite G or opposite A (Fig. 3). In our case the primary activity of APE1 is 6 orders of magnitude higher as compared to the exonuclease activity observed. Therefore, our data are mostly in agreement with the earlier conclusions concerning the enzymatic properties of APE1 [16,17] but are inconsistent with data published recently [19].

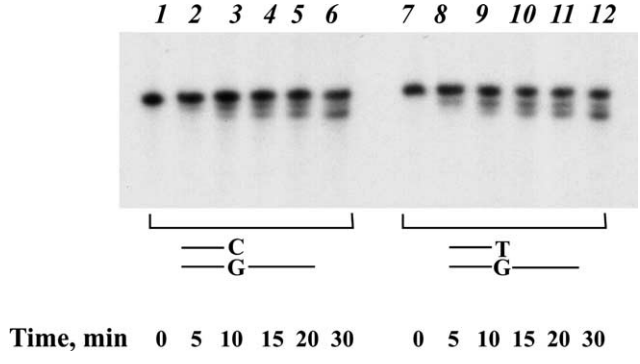

Fig. 4. Time dependence of $3' \rightarrow 5'$-exonucleolytic cleavage by APE1. APE1 was incubated with recessed matched CG (lanes 1–6) or mismatched TG (lanes 7–12) DNA structures. Concentrations of APE1 and DNA were 1 μM and 10 nM, respectively. The products were separated by denaturing PAGE followed by autoradiography.

Taking into account the importance of the conclusions we have compared in our experiments three different APE1 preparations purified independently in different laboratories. We found the same properties of APE1 for all three preparations, regardless of the purification procedures used.

The low $3' \rightarrow 5'$-exonuclease activity of APE1 revealed in our experiments has no strong preference for mismatched structures over matched ones. Time-course-dependent experiments using recessed DNA with matched (CG) and mismatched (TG) $3'$-terminus were carried out, however, we did not observe remarkable difference in the efficiency of exonuclease cleavage at high enzyme concentration (Fig. 4). To confirm the lack of specificity of APE1 exonuclease activity to mismatched structures we used another system. TMP moiety was incorporated by DNA polymerase β opposite A using recessed DNA structure and TTP as a substrate, followed by addition of APE1. We found that incorporated TMP moiety from matched base pair could be efficiently removed at high concentrations of APE1 (10^{-5}–10^{-6} M) (data not shown).

Discussion

The misincorporation of nucleotides during DNA replication or DNA repair processes results in mutagenesis. It is surprising that the fidelity of the key DNA polymerase of base excision repair, namely of DNA polymerase β, is very low. Taking into account an approximate estimation of 20,000 AP sites produced per cell per day [24], the DNA base excision repair system seems to be extremely mutagenic and certainly some powerful proofreading mechanisms are used. Several $3' \rightarrow 5'$-exonucleases have been discovered and suggested as candidates to provide proofreading function for BER [25–29]. One of them is TREX1 [26,27]. However, intracellular localization of TREX1 has not been determined clearly and TREX1 has only a minor preference for mismatched DNA in comparison to matched [26,27]. Therefore, the discovery of exonuclease activity associated with APE1 employing specifically mismatched structures seemed conceptually very important [30]. This enzyme localizes in the nucleus where BER takes place and is considered as a good candidate to cooperate with DNA polymerase β in the BER pathway [19]. It was shown that this exonuclease activity associated with APE1 has a preference for mismatched structures located at $3'$-terminus of upstream primer of nicked or gapped DNA. In contrast to earlier publications [16,17], it was suggested that this exonuclease activity could be regarded as biologically significant to provide BER fidelity mechanism. In addition it was found that the rejoining reaction is dependent on the presence of APE1 [19]. Therefore, it was suggested that

slow rejoining reaction should create the time window at this stage of BER pathways to remove mismatches by 3′ → 5′-exonuclease activity associated with APE1 [19].

In our opinion another scenario can happen. APE1 is not leaving AP site immediately after endonucleolytic cleavage prior to the lyase action of DNA polymerase β. Misincorporation of dNMP by DNA polymerase β can stimulate the 3′ → 5′-exonuclease activity of APE1 and mismatch can be removed before the lyase action of DNA polymerase operates. Along with that idea we examined 3′ → 5′-exonuclease activity of APE1 for recessed DNA and nicked, gapped DNA structures containing phosphate and sugar phosphate at the 5′-termini of downstream primers. We found that under the reaction conditions optimal for AP endonuclease activity the 3′ → 5′-exonuclease activity could be observed only if enzyme concentration was elevated by 6–7 orders of magnitude. This activity does not show a strong preference for mismatched over the matched structures and furthermore, this activity does not demonstrate specificity with respect to nicked or gapped as compared to recessed DNAs. The presence of analog of sugar phosphate moiety at 5′-terminus of downstream primer of nicked or gapped DNA structures demonstrates inhibition rather than stimulation of 3′ → 5′-exonuclease activity. These data are consistent with the preference of this structure to bind APE1 rather than contaminant exonuclease. Interaction of APE1 with nicked DNA containing tetrahydrofuranophosphate moiety at 5′-margin was shown previously [31,32].

Therefore, on the basis of our results we conclude that 3′ → 5′-exonuclease activity associated with APE1 is not likely to represent a key mechanism contributing to the fidelity of base excision repair. This activity is observable only at high enzyme concentration and thus one of the possible explanations can be that the traces of contaminant exonuclease could be copurified with APE1. On the other hand, it was shown that APE1 could efficiently remove anticancer nucleoside analogs having unnatural stereo chemical L-configuration as well as nucleosides with modified ribose [18]. The question still remains whether the primary endonuclease activity of the enzyme can be responsible for removing matched or mismatched nucleotides from 3′-termini under some specific conditions. At least at the conditions optimal for endonuclease cleavage 3′ → 5′-exonuclease activity of APE1 towards dNMP residues at 3′-termini is almost negligible.

Acknowledgments

The authors are grateful to Valerie Lefort for technical assistance. This work was supported by grants from the Russian Foundation for Basic Research (00-04-22002, 01-04-48895, and 02-04-48404), by PICS, by Projects[1] 12.1 of RAS, and by INTAS Grant YSF-00-83. Olga Lavrik was a recipient of a grant from Paris 6 University International Agreement (PAST).

References

[1] T. Lindahl, DNA repair enzymes, Annu. Rev. Biochem. 51 (1982) 61–87.
[2] T. Lindahl, R.D. Wood, Quality control by DNA repair, Science 286 (1999) 1897–1905.
[3] G. Frosina, P. Fortini, O. Rossi, F. Carrozzino, G. Raspaglio, L.S. Cox, D.P. Lane, A. Abbondandolo, E. Dogliotti, Two pathways for base excision repair in mammalian cells, J. Biol. Chem. 271 (1996) 9573–9578.
[4] Y. Kubota, R.A. Nash, A. Klungland, P. Schar, D.E. Barnes, T. Lindahl, Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase β and the XRCC1 protein, EMBO J. 15 (1996) 6662–6670.
[5] I.D. Nicholl, K. Nealon, M.K. Kenny, Reconstitution of human base excision repair with purified proteins, Biochemistry 36 (1997) 7557–7566.
[6] D.K. Srivastava, B.J. Berg, R. Prasad, J.T. Molina, W.A. Beard, A.E. Tomkinson, S.H. Wilson, Mammalian abasic site base excision repair. Identification of the reaction sequence and rate-determining steps, J. Biol. Chem. 273 (1998) 21203–21209.
[7] P.W. Doetsch, R.P. Cunningham, The enzymology of apurinic/apyrimidinic endonucleases, Mutat. Res. 236 (1990) 173–201.
[8] Y. Matsumoto, K. Kim, Excision of deoxyribose phosphate residues by DNA polymerase β during DNA repair, Science 269 (1995) 699–702.
[9] R. Prasad, R.K. Singhal, R.K. Srivastava, J.T. Molina, A.E. Tomkinson, S.H. Wilson, Specific interaction of DNA polymerase β and DNA ligase I in a multiprotein base excision repair complex from bovine testis, J. Biol. Chem. 271 (1996) 16000–16007.
[10] R.K. Singhal, R. Prasad, S.H. Wilson, DNA polymerase β conducts the gap-filling step in uracil-initiated base excision repair in a bovine testis nuclear extract, J. Biol. Chem. 270 (1995) 949–957.
[11] G. Dianov, C. Bischoff, J. Piotrowski, V.A. Bohr, Repair pathways for processing of 8-oxoguanine in DNA by mammalian cell extracts, J. Biol. Chem. 273 (1998) 33811–33816.
[12] A. Klungland, M. Hoss, D. Gunz, A. Constantinou, S.G. Clarkson, P.W. Doetsch, P.H. Bolton, R.D. Wood, T. Lindahl, Base excision repair of oxidative DNA damage activated by XPG protein, Mol. Cell 1 (1999) 33–42.
[13] R. Prasad, G.L. Dianov, V.A. Bohr, S.H. Wilson, FEN1 stimulation of DNA polymerase β mediates an excision step in mammalian long patch base excision repair, J. Biol. Chem. 275 (2000) 4460–4466.
[14] B.A. Sokhansanj, G.R. Rodrigue, J.P. Fitch, D.M. Wilson III, A quantitative model of human DNA base excision repair. I. Mechanistic insights, Nucleic Acids Res. 30 (2002) 1817–1825.
[15] G.L. Dianov, R. Prasad, S.H. Wilson, V.A. Bohr, Role of DNA polymerase β in the excision step of long patch mammalian base excision repair, J. Biol. Chem. 274 (1999) 13741–13743.
[16] B. Demple, T. Herman, D.S. Chen, Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: definition of a family of DNA repair l, Proc. Natl. Acad. Sci. USA 88 (1991) 11450–11454.
[17] C.N. Robson, A.M. Milne, D.J. Pappin, I.D. Hickson, Isolation of cDNA clones encoding an enzyme from bovine cells that repairs oxidative DNA damage in vitro: homology with bacterial repair enzymes, Nucleic Acids Res. 19 (1991) 1087–1092.
[18] K.M. Chou, M. Kukhanova, Y.C. Cheng, A novel action of human apurinic/apyrimidinic endonuclease: excision of L-configuration deoxyribonucleoside analogs from the 3′ termini of DNA, J. Biol. Chem. 275 (2000) 31009–31015.
[19] K.M. Chou, Y.C. Cheng, An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3′ mispaired DNA. An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3′ mispaired DNA, Nature 415 (2002) 655–659.

[20] R.A. Bennett, D.M. Wilson III, D. Wong, B. Demple, Interaction of human apurinic endonuclease and DNA polymerase β in the base excision repair pathway, Proc. Natl. Acad. Sci. USA 94 (1997) 7166–7169.
[21] I.A. Drachkova, I.O. Petruseva, I.V. Safronov, A.L. Zakharenko, G.V. Shishkin, O.I. Lavrik, S.N. Khodyreva, Reagents for modification of protein–nucleic acids complexes. II. Site-specific photomodification of DNA-polymerase β complexes with primers elongated by the dCTP exo-N-substituted arylazido derivatives, Bioorg. Khim. Moscow 27 (2001) 197–204.
[22] P.R. Strauss, W.A. Beard, T.A. Patterson, S.H. Wilson, Substrate binding by human apurinic/apyrimidinic endonuclease indicates a Briggs–Haldane mechanism, J. Biol. Chem. 272 (1997) 1302–1307.
[23] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.
[24] T. Lindahl, Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair, Mutat. Res. 462 (2000) 129–135.
[25] M. Hoss, P. Robins, T.J. Naven, D.J. Pappin, J. Sgouros, T. Lindahl, A human DNA editing enzyme homologous to the *Escherichia coli* DnaQ/MutD protein, EMBO J. 18 (1999) 3868–3875.
[26] D.J. Mazur, F.W. Perrino, Identification and expression of the TREX1 and TREX2 cDNA sequences encoding mammalian $3' \rightarrow 5'$ exonucleases, J. Biol. Chem. 274 (1999) 19655–19660.
[27] D.J. Mazur, F.W. Perrino, Excision of 3′ termini by the Trex1 and TREX2 $3' \rightarrow 5'$ exonucleases. Characterization of the recombinant proteins, J. Biol. Chem. 276 (2001) 17022–17029.
[28] I.V. Shevelev, N.V. Belyakova, T.P. Kravetskaya, V.M. Krutyakov, Autonomous $3' \rightarrow 5'$ exonucleases can proofread for DNA polymerase β from rat liver, Mutat. Res. 459 (2000) 237–242.
[29] I.V. Shevelev, U. Hubscher, The 3′5′ exonucleases, Nat. Rev. Mol. Cell Biol. 3 (2002) 1–12.
[30] J. Jiricny, An APE that proofreads, Nature 415 (2002) 593–594.
[31] O.I. Lavrik, R. Prasad, R.W. Sobol, J.K. Horton, E.J. Ackerman, S.H. Wilson, Photoaffinity labeling of mouse fibroblast enzymes by a base excision repair intermediate. Evidence for the role of poly(ADP-ribose) polymerase-1 in DNA repair, J. Biol. Chem. 276 (2001) 25541–25548.
[32] T.A. Ranalli, S. Tom, R.A. Bambara, AP endonuclease 1 coordinates flap endonuclease 1 and DNA ligase I activity in long patch base excision repair, J. Biol. Chem. 277 (2002) 41715–41724.