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Effect of multifunctional protein YB-1 on the AP site cleavage by AP endonuclease 1 and tyrosyl phosphodiesterase 1

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Apurinic/apyrimidinic sites (AP sites) which represent one of the most abundantly generated DNA lesions in the cell are generally repaired by base excision repair (BER) pathway. Multifunctional protein YB-1 is known to participate in cellular response to genotoxic stress and was shown to interact with several components of BER – DNA glycosylases NTH1, NEIL2, DNA polymerase β and DNA ligase III. Therefore, it is of great interest to investigate the influence of YB-1 on one of the major BER enzymes, responsible for AP site cleavage, AP endonuclease APE1, and on tyrosyl phosphodiesterase Tdp1, participating in APE1 independent pathway of AP site repair. ***Aim****. Effect of multifunctional protein YB-1 on the AP site cleavage by the activities of APE1 and Tdp1 was studied.* ***Methods****. Gel-mobility shift assays and enzyme activity tests.* ***Results****. YB-1 was shown to inhibit the cleavage of AP site located in single-stranded DNA by both APE1 and Tdp1. Stimulation of APE1 activity on protruding double-stranded DNA in the presence of YB-1 was observed, whereas no effect on Tdp1-mediated cleavage of AP site in double-stranded DNA was found.* ***Conclusions****. YB-1 can modulate the repair of AP sites in DNA by both positively stimulating APE1 during the classic BER of AP sites and avoiding a possible generation of double-strand breaks, arising from the cleavage of single-stranded portion of DNA substrate already used by different DNA-processing pathway.*

Keywords: AP site repair, AP endonuclease 1, tyrosyl phosphodiesterase 1, YB-1.

Introduction. AP sites in DNA appear as a result of release of the nucleobase from the nucleotide residue in DNA. The process is going both spontaneously and as DNA glycosylase-driven release of damaged nucleobases repaired by base excision repair (BER) of DNA. AP sites are also representative DNA damages, since absence of coding base in DNA leads to the blockade of replicative DNA polymerases or error-prone TLS synthesis across the lesion [1], which in turn define cytotoxicity and mutations. The major repair pathway of AP sites is BER which consists in the cleavage of DNA, containing AP site, followed by DNA repair synthesis and ligation. The major AP site cleaving activity is attributed to APE1, however, there is evidence of APE1 independent pathway of repair, which utilize the AP lyase activity of bifunctional DNA glycosylases such as NEIL2 or NTH1 and tyrosyl phosphodiesterase Tdp1 [2, 3]. In recent studies it was shown that Tdp1 can initiate the repair of AP sites *in vitro*, cleaving DNA at the position of AP site [4]. Such pathways can contribute to the backup for APE1-dependent AP site repair. One of the proteins that can affect both the mainstream and backup ways of the repair of AP sites is multifunctional Y-box binding protein YB-1. YB-1 directly interacts with en-

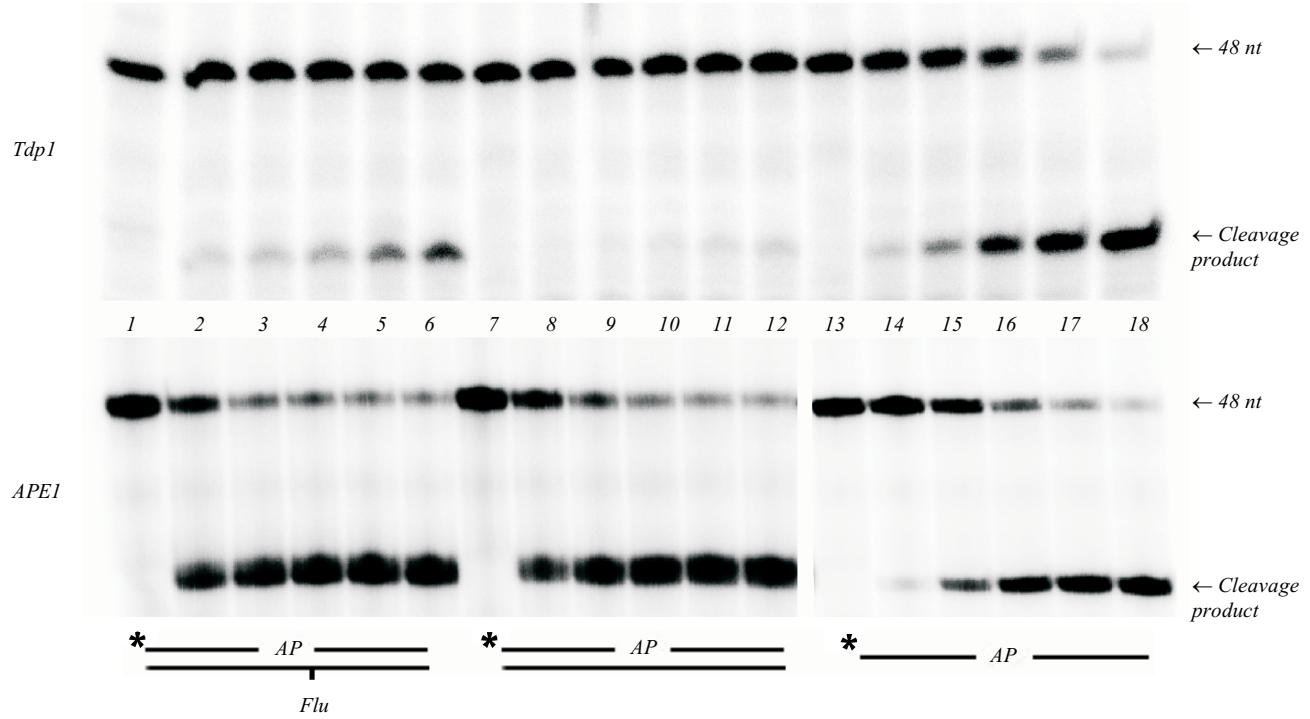

Fig. 1. Substrate specificity of Tdp and APE1. Incubation time (min): *1* – 0; *2* – 2; *3* – 5; *4* – 10; *5* – 20; *6* – 40; *7* – 0; *8* – 2; *9* – 5; *10* – 10; *11* – 20; *12* – 40; *13* – 0; *14* – 2; *15* – 5; *16* – 10; *17* – 20; *18* – 40

zymes responsible for the initial stages of BER – APE1, NEIL2 and NTH1, stimulating the activities of the latter two enzymes, when the repair of oxidatively damaged DNA is performed (reviewed in [5]). We also have shown recently that YB-1 modulates AP lyase activity of bifunctional DNA glycosylase/AP lyase NEIL1 [6]. Therefore, the aim of present study was to investigate the influence of YB-1 on the other enzymes responsible for the AP site cleavage during the repair of AP sites – APE1 and Tdp1.

Materials and methods. The following proteins and reagents were used: *Escherichia coli* Udg, phage T4 polynucleotide kinase («Biosan», Russia); [γ-^{32}P] ATP with specific activity 5000 Ci/mmol (LBT ICBFM SB RAS); reagents for electrophoresis in polyacrylamide gel and the main buffers components («Sigma», USA). Recombinant YB-1 was purified from *E. coli* BL21 (DE3) cells as described in [7]. Purified recombinant Tdp1 was kindly provided by N. A. Lebedeva (LBCE ICBFM SB RAS), human recombinant APE1 was a generous gift from S. N. Khodyreva (LBCE ICBFM SB RAS). The following oligonucleotides were used: damaged strand 5'-(d)CTAT GGCG AGGC GATT AAGT TGGG ***U***AAC GTCA GGGT CTTC CGAA CGAC-3', complementary strand 5'-(d)GTCG TTCG GAAG ACCC TGAC GTTG CCCA ACTT AATC GCCT CGCC ATAG-3', complementary strand, containing fluorescein-dUMP residue instead of dGMP residue (underlined) in 24 position from 5'-end («Nanotech-C», Russian Federation), 60-mer and 32-mer oligonucleotides with sequences identical to those published in [6]. 5'-^{32}P-labelling of the dUMP-containing oligonucleotide, annealing to complementary strand and *in situ* generation of AP sites were performed essentially as in [6].

Enzymatic reactions and GMSA experiments were performed in 50 mM sodium phosphate buffer, pH 7.8, 100 mM NaCl, 2 mM EDTA, 0.1 g/l BSA, 0.2 mM DTT, 2.5 % glycerol and in case of APE1 5 mM $MgCl_2$. Enzymes (Tdp1 at final concentration 200 nM, APE1 at 0.3 nM and 0.6 nM for dsDNA and ssDNA experiments, respectively), DNA (50 nM final concentration) and YB-1 (if used) were incubated for indicated periods of time or 20 min at 37 °C. Quenching of the reaction and analysis of reaction products were performed essentially as in [6].

Gel-mobility shift assays (GMSA) experiments were performed essentially as in [6].

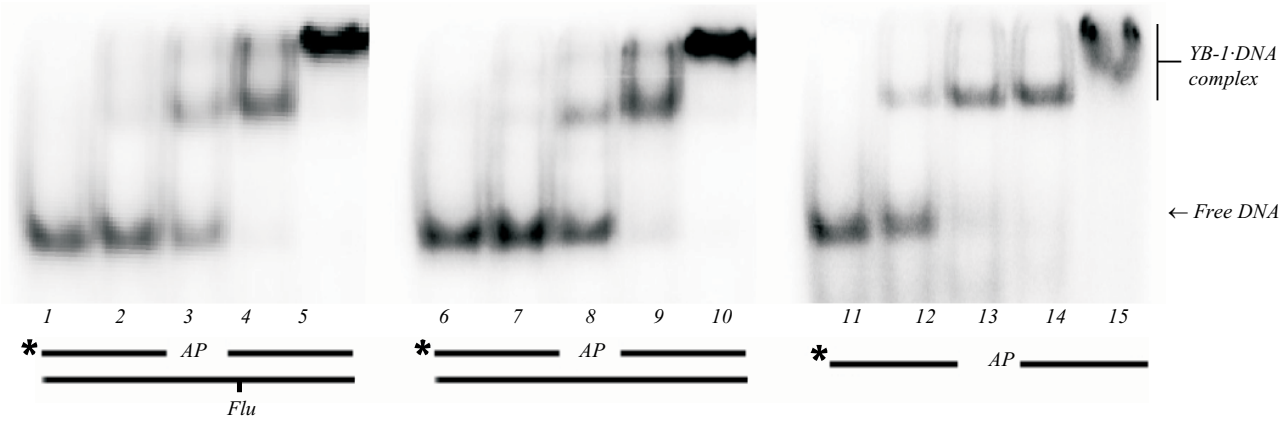

Fig. 2. Affinity of YB-1 towards AP site containing DNA. YB-1 concentration in nM and (percentage of DNA bound, %): *1* – 0 (0); *2* – 50 (18); *3* – 125 (53); *4* – 250 (100); *5* – 500 (100); *6* – 0 (0); *7* – 50 (10); *8* – 125 (41); *9* – 250 (95); *10* – 500 (100); *11* – 0 (0); *12* – 50 (28); *13* – 125 (74); *14* – 250 (100); *15* – 500 (100)

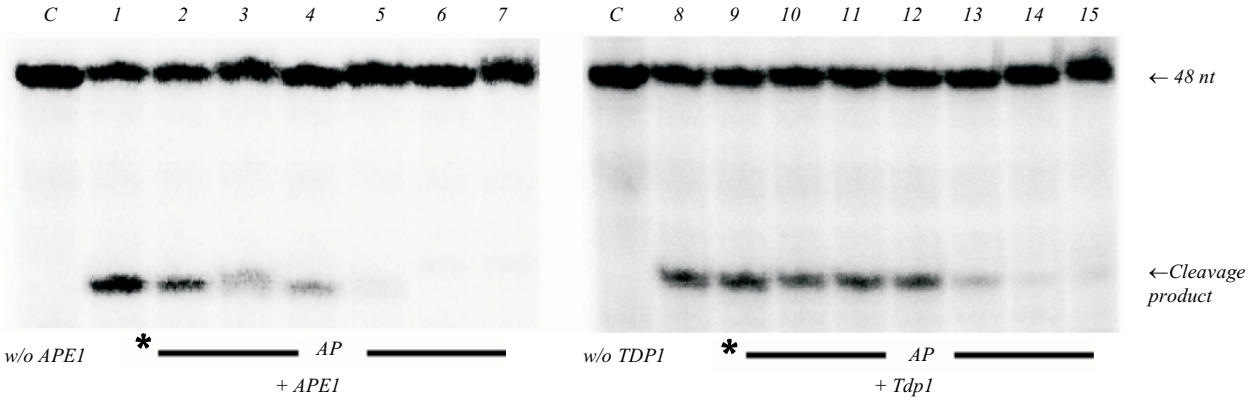

Fig. 3. Inhibition of AP site cleavage by APE1 and Tdp1 in the presence of YB-1. *C* – control experiments, lacking APE1 and Tdp1. YB-1 concentration in nM: *1* – 0; *2* – 12.5; *3* – 25; *4* – 50; *5* – 125; *6* – 250; *7* – 500; *8* – 0; *9* – 5; *10* – 12.5; *11* – 25; *12* – 50; *13* – 125; *14* – 250; *15* – 500

Results and discussion. We assayed AP site cleavage activity of APE1 and Tdp1 on three different AP site-containing substrates – double-stranded DNA, single-stranded DNA and double-stranded DNA, containing fluorescein residue in + 1 position in the complementary strand opposite the AP site (Fig. 1). The latter substrate imitates DNA, bearing clustered lesions that require different DNA repair pathways.

We have found that despite the mechanistic model depicting physical interaction of APE with nucleotides adjacent to the AP site [8], presence of bulky fluorescein residue in close proximity to AP site does not affect endonucleolytic activity of enzyme. Similarly with earlier observations we reveal that introduction of bulky moiety in close proximity to AP site in double-stranded DNA transforms such DNA to a much better substrate for Tdp1. Results from GMSA experiments suggest that YB-1 affinity towards DNA, containing AP site clustered with bulky lesion, is higher than to DNA with single AP site, however under reaction conditions used the highest affinity of YB-1 was shown for the single-stranded DNA substrate (Fig. 2). Addition of YB-1 to the reaction mixture affected both APE1 and Tdp1 activities, depending on the DNA substrate used (Fig. 3, 4). Evident inhibition of the AP site cleavage located in the single-stranded DNA was observed for both enzymes (Fig. 3). The same inhibitory effect was shown, when single-stranded AP site-containing DNA of two other lengths – 32 and 60 nt were tested (data not shown). Such findings are consistent with published data showing negative regulation of NEIL1 AP lyase activity by YB-1 when single-stranded DNA was used [6].

On the other hand, almost no effect was found when APE1 or Tdp1 activity was assayed in reaction mixtures, containing double-stranded 48-mer DNA substrates with clustered lesion or single AP site, that were

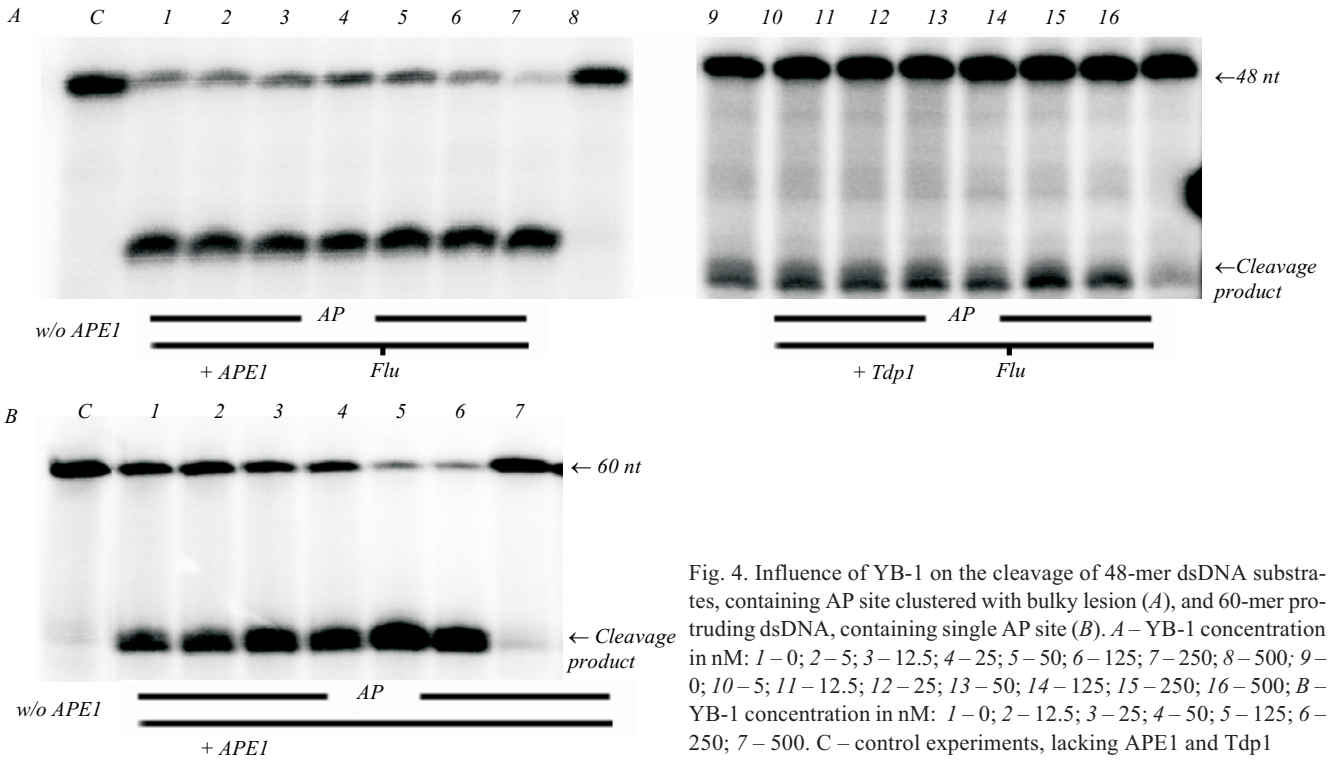

Fig. 4. Influence of YB-1 on the cleavage of 48-mer dsDNA substrates, containing AP site clustered with bulky lesion (*A*), and 60-mer protruding dsDNA, containing single AP site (*B*). *A* – YB-1 concentration in nM: *1* – 0; *2* – 5; *3* – 12.5; *4* – 25; *5* – 50; *6* – 125; *7* – 250; *8* – 500; *9* – 0; *10* – 5; *11* – 12.5; *12* – 25; *13* – 50; *14* – 125; *15* – 250; *16* – 500; *B* – YB-1 concentration in nM: *1* – 0; *2* – 12.5; *3* – 25; *4* – 50; *5* – 125; *6* – 250; *7* – 500. C – control experiments, lacking APE1 and Tdp1

supplemented with YB-1 up to certain concentration (Fig. 4, *A*, and data not shown).

Similarly absence of functional interaction between YB-1 and APE1 was reported earlier in experiments with 43-mer double-stranded substrates, when YB-1 was in shortage in comparison to the amount of DNA [9]. Under our experimental conditions, however, large (10-fold) molar excess of YB-1 over DNA lead to almost complete inhibition of AP site cleavage. Taking together with YB-1 ability to form multimers [10], data from GMSA experiments (Fig. 1) and NaBH4-mediated crosslinking of YB-1 directly to AP site [6] we assume that at such high concentrations several YB-1 molecules are bound to the DNA substrate in specific conformation forming multimeric complex. Such complex is stabilized by Schiff base formation of primary amino group of protein with aldehyde group of AP site which presumably results in blocking the access of APE1 or Tdp1 to the AP site.

Stimulation of APE1 activity by YB-1 on double-stranded DNA was observed when longer 60-mer AP site-containing DNA substrate was used (Fig. 4, *B*). These data are in agreement with our previous observations that YB-1 notably stimulates AP lyase activity of NEIL1 when protruding double-stranded substrate was used and may reflect specific interaction of YB-1 with such DNA substrate.

Taken together the data from present and previous studies imply that YB-1 can play a modulatory role during the repair of AP sites in DNA both by positively stimulating the cleavage of AP site located in specific double-stranded substrates and by avoiding the cleavage of single-stranded portion of unwound DNA substrate, which is already used by different DNA-processing pathway (i. e. DNA replication, transcription or different types of repair). YB-1 can affect both mainstream and back-up pathways influencing major AP endonuclease – APE1 and enzymes that can serve as a backup for AP site cleavage reaction – bifunctional glycosylase NEIL1 and tyrosyl phosphodiesterase Tdp1. Involvements of YB-1 itself into abovementioned DNA-processing events (transcription activity of YB-1, putative role of YB-1 in DNA replication and repair (reviewed in [5])) would serve as a basis for coordinating these processes with the repair of AP sites.

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Вплив мультифункціонального білка YB-1 на розщеплення АП-сайтів АП-ендонуклеазою 1 і тирозил-ДНК-фосфодіестеразою 1

Резюме

Апуринові/апіримідинові сайти (АП-сайти), які є одними з найчисельніших пошкоджень ДНК у клітині, репаруються, в основному, шляхом ексцизійної репарації основ (ЕРО). Багатофункціональний білок YB-1 бере участь у клітинній відповіді на генотоксичний вплив і взаємодіє з деякими компонентами системи ЕРО – ДНК-глікозилазами NTH1, NEIL2, ДНК-полімеразою β і ДНК-лігазою III. Безсумнівний інтерес становить вивчення дії YB-1 на один з ключових ферментів ЕРО, який відповідає за розщеплення АП-сайтів, – АП-ендонуклеазу 1 (APE1), а також на тирозил-ДНК-фосфодіестеразу 1 (Tdp1), що причетна до APE1-незалежного шляху репарації АП-сайтів. ***Мета****. Вивчення впливу багатофункціонального білка YB-1 на розщеплення АП-сайтів, каталізоване APE1 і Tdp1.* ***Методи****. Метод «затримки в гелі», аналіз ферментативної активності.* ***Результати****. Показано, що YB-1 інгібує каталізоване APE1 і Tdp1 розщеплення сАП-сайтів, розташованих в одноланцюговій ДНК. Виявлено, що YB-1 стимулює активність APE1 при розщепленні протяжного дволанцюгового ДНК-субстрата і не впливає на Tdp1-опосередковане розщеплення дволанцюгових АП-вмісних ДНК.* ***Висновки****. YB-1 здатний модулювати репарацію АП-сайтів у ДНК, з одного боку, стимулюючи APE1 при відновленні цілісності ДНК за «класичним» шляхом ЕРО, та, з другого боку, інгібуючи активність APE1 і Tdp1 на одноланцюгових ДНК та допомагаючи клітині уникнути можливого виникнення дволанцюгових розривів.*

Ключові слова: репарація АП-сайтів, АП-ендонуклеаза 1, тирозилфосфодіестераза 1, білок YB-1.

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Влияние мультифункционального белка YB-1 на расщепление АП-сайтов АП-эндонуклеазой 1 и тирозил-ДНК-фосфодиэстеразой 1

Резюме

Апуриновые/апиримидиновые сайты (АП-сайты), являющиеся одними из наиболее многочисленных повреждений ДНК в клетке, репарируются, в основном, по пути эксцизионной репарации оснований (ЭРО). Многофункциональный белок YB-1 участвует в клеточном ответе на генотоксические воздействия и взаимодействует с некоторыми компонентами системы ЭРО – ДНК-гликозилазами NTH1, NEIL2, ДНК-полимеразой β и ДНК-лигазой III. Несомненный интерес представляет изучение влияния YB-1 на один из ключевых ферментов ЭРО, ответственный за расщепление АП-сайтов, – АП-эндонуклеазу 1 (APE1), а также на тирозил-ДНК-фосфодиэстеразу 1 (Tdp1), участвующую в APE1-независимом пути репарации АП-сайтов. ***Цель****. Изучение влияния мультифункционального белка YB-1 на расщепление АП-сайтов, катализируемое APE1 и Tdp1.* ***Методы****. Метод «задержки в геле», анализ ферментативной активности.* ***Результаты****. Показано, что YB-1 ингибирует катализируемое APE1 и Tdp1 расщепление АП-сайтов, расположенных в одноцепочечной ДНК. Обнаружено, что YB-1 стимулирует активность APE1 при расщеплении протяженного двухцепочечного ДНК-субстрата и не влияет на Tdp1-опосредованное расщепление двухцепочечных АП-содержащих ДНК.* ***Выводы****. YB-1 способен модулировать репарацию АП-сайтов в ДНК, с одной стороны, стимулируя APE1 при восстановлении целосности ДНК по «классическому» пути ЭРО, и, с другой стороны, ингибируя активность APE1 и Tdp1 на одноцепочечных ДНК и помогая клетке избежать возможного образования двухцепочечных разрывов.*

Ключевые слова: репарация АП-сайтов, АП-эндонуклеаза 1, тирозилфосфодиэстераза 1, белок YB-1.

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