Cloning and Characterization of a Wheat Homologue of Apurinic/Apyrimidinic Endonuclease Ape1L

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Running title: *Characterization of the wheat AP endonuclease*

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Author Contributions: [g]The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

Supporting Information Legend

Supporting Information Figure Legends

Supporting Information Figure S1. SDS-PAGE analysis of the purified recombinant TaApe1 protein. Lane 1, protein size markers; lane 2, human APE1, 2 µg; lane 3, TaApe1, 1 µg; lane 4, TaApe1, 5 µg.

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Supporting Information Figure S3. Divalent cation dependence of wheat TaApe1-catalyzed activities on the oligonucleotide duplex THF•T. 10 nM 5'-^{32}P-labelled 30-mer DNA duplex containing a single THF residue was incubated for 10 min at 23°C with 5 or 10 nM TaApe1 under standard reaction conditions but in the presence of different divalent cations. (**A**) Effects of $MgCl_2$, $CaCl_2$ and $CoCl_2$ on the enzyme activities (**B**) Effects of $ZnCl_2$, $NiCl_2$ and $FeCl_2$ on the enzyme activities. Lanes 1, 3, 7, 11, control 30-mer duplex THF•T incubated in reaction buffer without enzyme; lane 2, as lane 1 but incubated with 1 nM APE1 for 5 min at 37°C; lanes 4–6, 8–10 and 12–14, 30-mer duplex THF•T incubated with TaApe1L in the presence of the indicated metal chloride. For details, see Materials and Methods.

Supporting Information Figure S4. Effects of the absence of divalent cations and/or presence of 1 mM EDTA in the reaction buffer on the TaApe1L AP endonuclease activity. 10 nM 5'-^{32}P-labelled 30-mer DNA duplex containing a single THF residue was incubated with 5 nM TaApe1L at 23°C. Lane 1, control non-treated THF•T duplex; lane 2, as lane 1 but incubated for 5 min at 37°C with 1 nM APE1; lane 3, as lane 2 but incubated with 5 nM TaApe1L and 1 mM $MnCl_2$ for 5 min at 23°C; lane 4, as lane 3 but incubated with 10 nM TaApe1L: lane 5, as lane 3 but incubated with 0 mM $MnCl_2$; lane 6, as lane 5 but incubated with 10 nM TaApe1L; lane 7, as lane 3 but incubated with 1 mM EDTA; lane 8, as lane 7 but incubated with 10 nM TaApe1; lane 9, as lane 3 but incubated with 5 mM EDTA; lane 10, as lane 9 but incubated with 10 nM TaApe1. The arrows mark the position of the 30-mer DNA substrate and 10-mer cleavage product. For details, see Materials and Methods.

Supporting Information Figure S5. Preparation of the DNA substrates containing a nick flanked with 3'-PA or 3'-OH termini or a 1-nt gap flanked with 3'-P to measure 3'-repair phoshodiesterase activities of TaApe1L. (**A**) Schematic representation of the structures of 3' and 5' DNA termini at a DNA nick or gap. (**B**) Separation of 5'-^{32}P-labelled oligonucleotide fragments by denaturing PAGE. 10 nM 5'-^{32}P-labelled 34-mer DNA duplex containing a single U•G base pair was incubated for 5 min at 37°C with 10 nM hUNG and then with either 20 nM Fpg or 20 nM Nth or 20 nM Nfo for 15 min at 37°C. Lane 1, control non-treated 34-mer duplex U•G; lane 2, as lane 1 but incubated with 10 nM hUNG and 20 nM Fpg; lane 3, as lane 1 but incubated with 10 nM hUNG and 20 nM Nth; lane 4, as lane 1 but incubated with 10 nM hUNG and 20 nM Nfo. The arrows mark the position of the full-length 34-mer substrate and 19-mer cleavage fragments containing by 3'-PA, 3'-OH and 3'-P. For details, see Materials and Methods.

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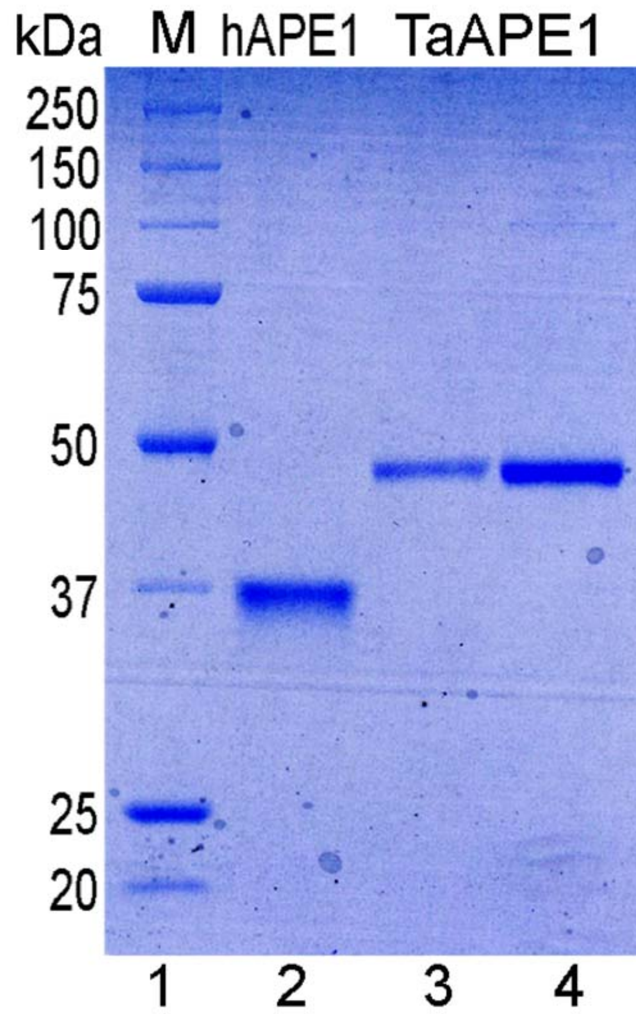

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AtArp     MNNALQFGLQSSAIYVAKFLVVPLRSLRVGSSFVGVGVGTRSFNKRLMSNATAFSINNSKRKELNIPGAAIDQNCHQMGSDTDRDEMGTLQDDRKEIEAM  100

AtArp     TVQELRSTLRKLGVPVKGRKQELISTLRLHMDSNLPDQKETSSSTRSDSVTIKRKISNREEPTEDECTNSEAYDIEHGEKRVKQSTEKNLKAKVSAKAIA  200
APE1      ----------------------------------------------------------------------------MPKRGKKGAV--AEDGD-ELR---   18

AtApe2    ----------------------------------------------------------------------------MKIVTYNVNGLRQRVSQFD-SLLK   23
AtApe1L   ------MKRFFKPIEKEN---SPAA------------KKPCLS------------PE-KRDGDGDGVEEEKNQNEPSKFMTWNANSFLLRVKNDWSQFSK   66
AtArp     KEQKSLMRTGKQQI-QSKEETSSTISSKLLKTEEIISSPSQSEPWTVLAHKKPQKDWKAYNPKTMRPPPLPEGTKCVKVMTWNVNGLRGLLKFESFSALQ  299
APE1      ------------TEPEAKKSKTAAKK--------------------NDKEAAG-EGPALYEDPPDQKTSPSGKPATLKICSWNVDGLRAWIKKKG---LD   82
                                                                                       *. ::*.:.:   :.       .

AtApe2    LLDSFDADIICFQETKLRRQELT-----------------ADL--AI-------ADGYESFFSCTRTSEKGRTGYSGVATFCRVKSASSSCETALPVTAE   97
AtApe1L   FVSDFDPDVIAIQEVRMPAAGGKGKPKNHEELSDDTKVLREEKQILTRALSSPPFGNYGVWWSLA------DSKYAGTALLVKKCFK--------PRKVY  152
AtArp     LAQRENFDILCLQETKLQVKDVE-----------------EIKKTLI-------DGYDHSFWSCS----VSKLGYSGTAIISR--IK--------PLSVR  361
APE1      WVKEEAPDILCLQETKCSENKLP-----------------AEL-QEL-------PGLSHQYWSAP----SDKEGYSGVGLLSR--QC--------PLKVS  143
            .    *::.:**.:                                       .    ::* .         *:*.. : :            * ..

AtApe2    EGITGLVNSNSRGGKSETSTVAEGLEEYEKEELLMIDQEGRCVITDHGHFVVFNVYGPRAVADDADRIEFK--HRFYGVLERRWECLLRQGRRVFVVGDL  195
AtApe1L   FNLD-----------------------KLA---SKHEPDGRVILAEFETFRLLNTYSPNNGWKDE-ENAFQRRRKWDKRIVEFLN--KTSDKPLIWCGDL  223
AtArp     YGTG-----------------------LSG---SDHDTEGRIVTAEFDSFYLINTYVPNSGDGLK-RLSYRI-EEWDRTLSNHI-KELEKSKPVVLTGDL  432
APE1      YGIG-----------------------DE-----EHDQEGRVIVAEFDSFVLVTAYVPNAGRGLV-RLEYR--QRWDEAFRKFL-KGLASRKPLVLCGDL  211
           .                                  : :** : ::.  * :...* *.       .  ::  ..:   : .       . : :.  ***

AtApe2    NIAPFAMDRCE--A------------------GPDFEKNEFRKWFRSLLVERGGSFSDVFRSKHPERK--DAFTCWSSSSGAEQFNYGSRIDHILVAGSC  273
AtApe1L   NVSHEEIDVSHPEFFATAKLNGYVPPNKEDCGQPGFTP-SERGRFGATIKE--GRLVDAYRYLHKEQEMESGFSWSG-NPIGKYRGKRMRIDYFLVSEQL  319
AtArp     NCAHEEIDIFNPAGNK-R--------------SAGFTE-EERQSFGANFLD--KGFVDTFRKQHPGV---VGYTYWGYRHGGRKTNKGWRLDYFLVSQSI  511
APE1      NVAHEEIDLRNPKGNK-K--------------NAGFTP-QERQGFGELLQA--VPLADSFRHLYPNTP--YAYTFWTYMMNARSKNVGWRLDYFLLSHSL  291
          * :   :*  .                      ..*   . *  *   :      : * :*  :       .::       ..  .   *:*::*:: .

AtApe2    LHQDEDKQGHSFLACHVKECDILTEYKRFKNENMPTRWKGGLVTKFKGSDHVPVFISFDDLPDIPEHSTP-PLASRYLPMIYGFQQTLVSVFKKRRANEE  372
AtApe1L   KDRIVSCKM---------------------------HGRGIELEGFHGSDHCPVTLELSKPSSEMEQNQVSN                              364
AtArp     AANVHDS-------------------------------Y--ILPDINGSDHCPIGLILKL                                          538
APE1      LPALCDS-------------------------------K--IRSKALGSDHCPITLYLAL                                          318
               .                                         **** *: : :

AtApe2    AKAIEVSCSSSTQSNTSSICGDISTGPLRNCGSMGISLEKSCSFENKSTSGVTEAETVAATGSIDNLSDGIRASSVRALNISRDGDRKKARKIQSSQLSL  472

AtApe2    KSFFTTNSKVNNVEDSSSSYVSSSPSSQVESITEPNVSGKEDSEPTTSTQEQDQTGSSAKQKNDAALMEWQRIQNLMQNSIPLCKGHKEACVARVVKKPG  572

AtApe2    PTFGRRFYVCSRAEKQTVVISNGLHQNSETSKRVMRSK                                                                610
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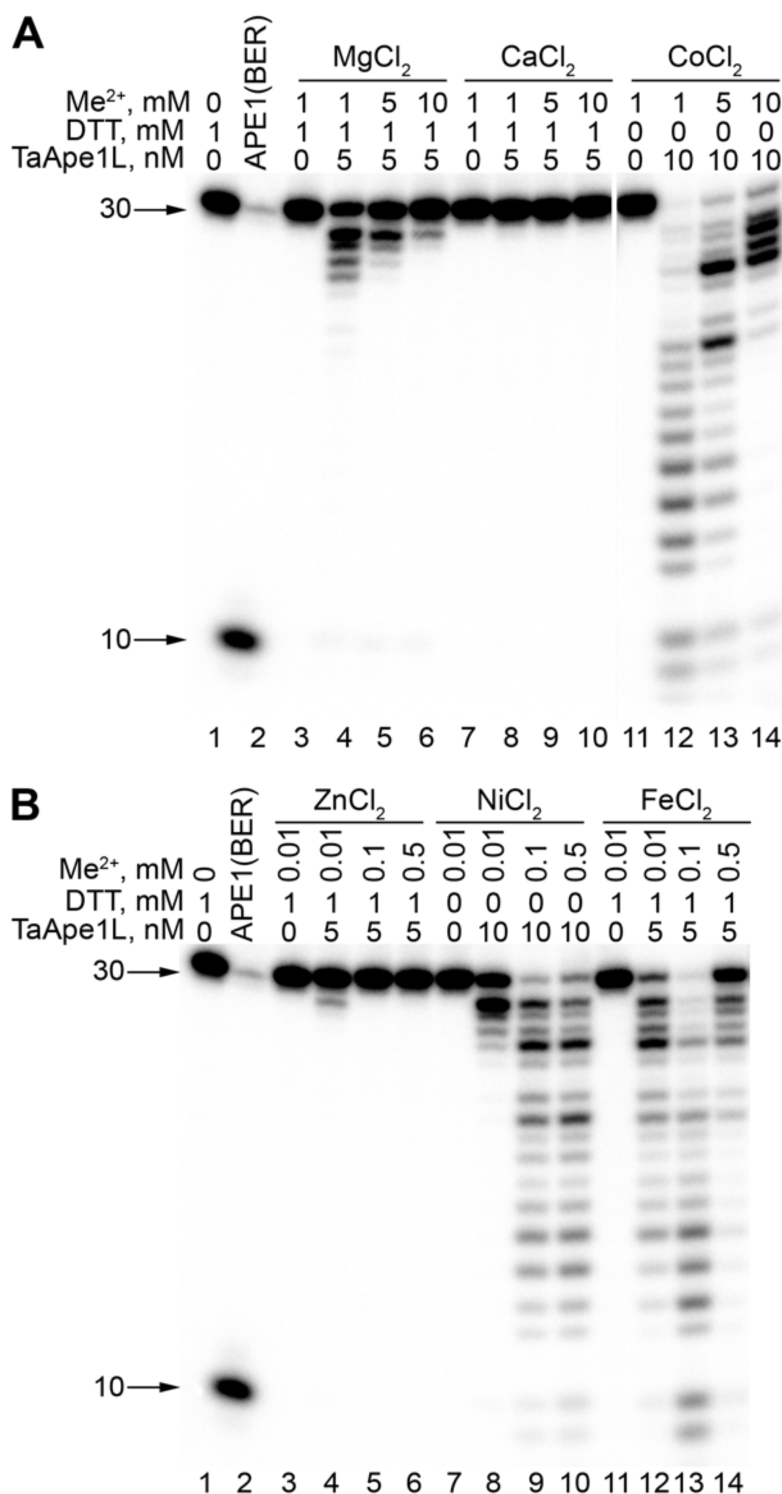

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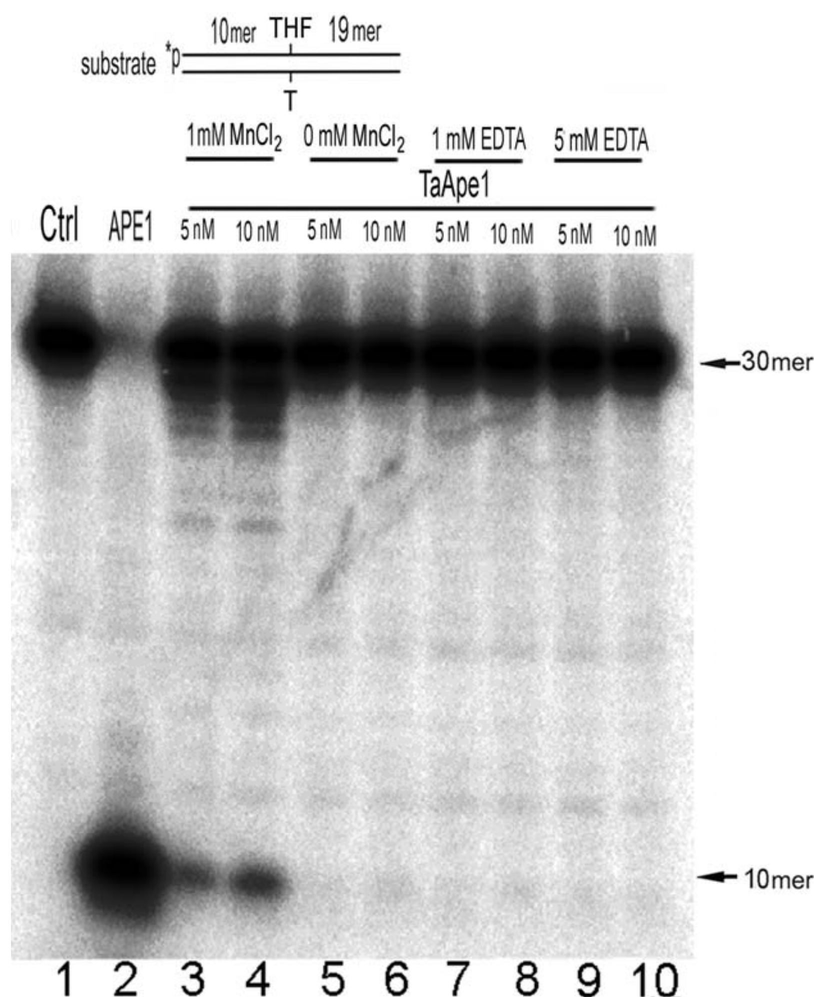

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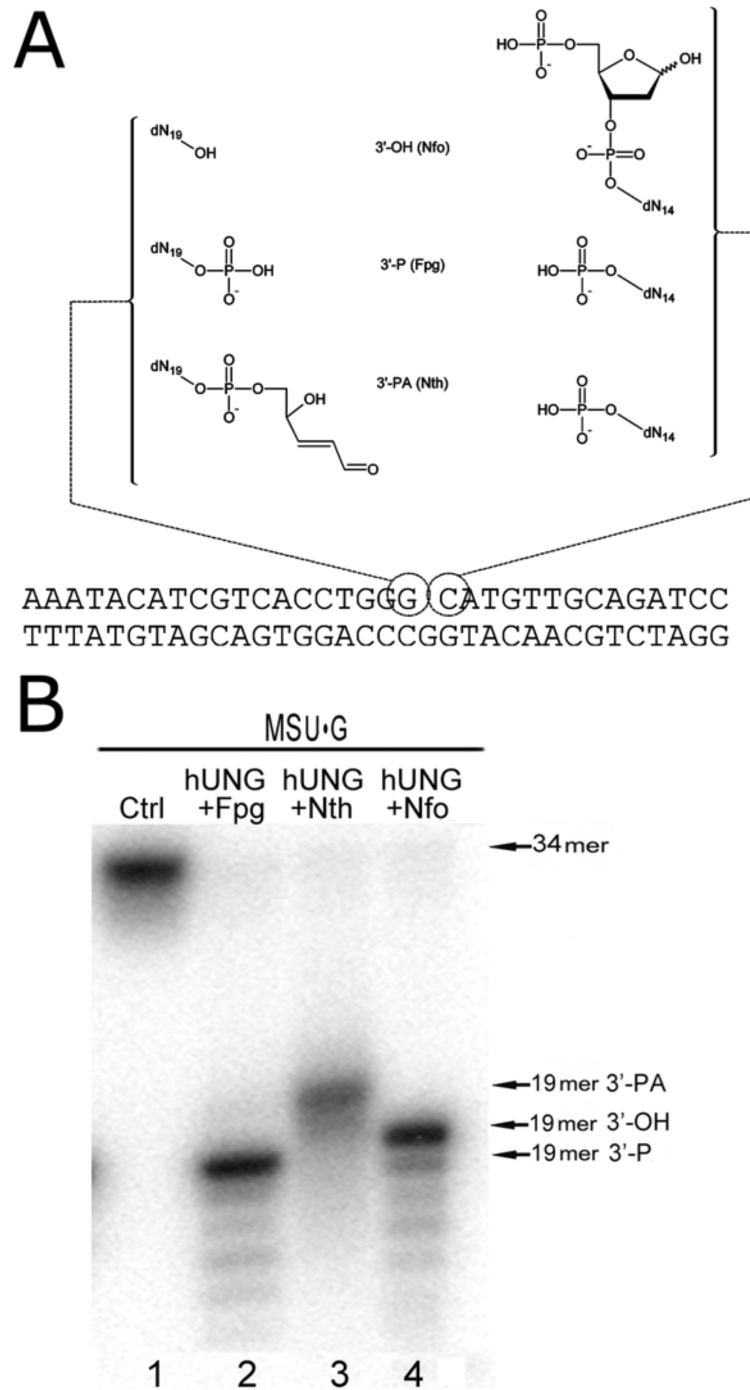

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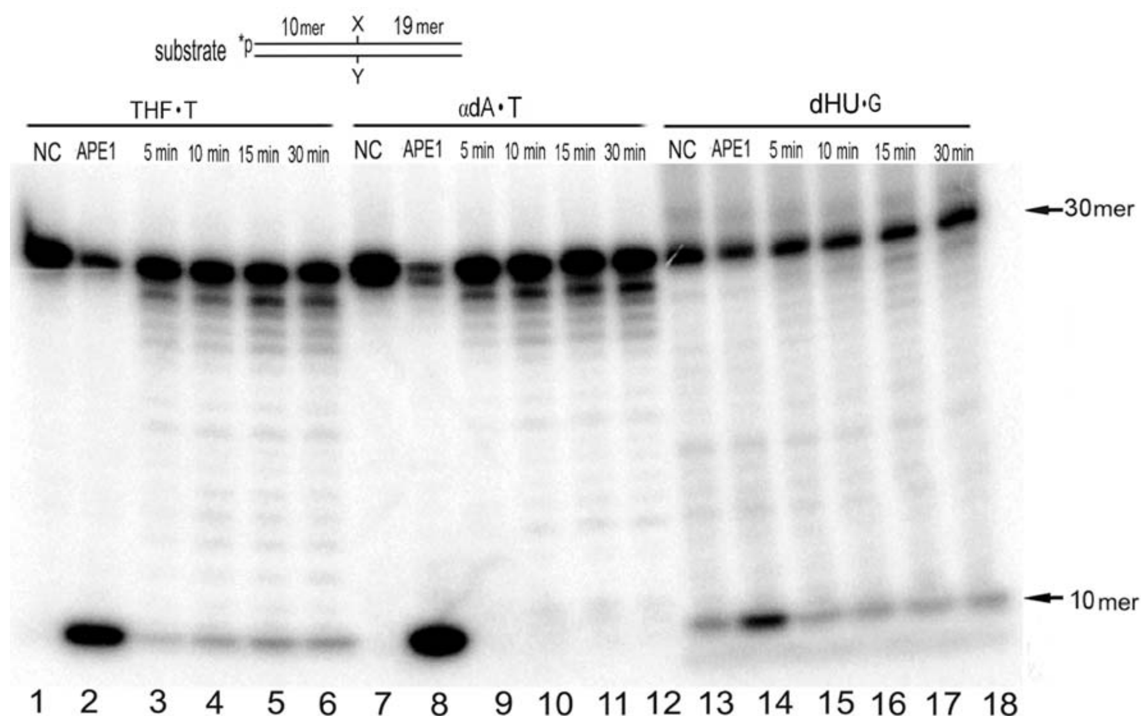

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