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The role of *Schizosaccharomyces pombe* DNA repair enzymes Apn1p and Uve1p in the base excision repair of apurinic/apyrimidinic sites ☆

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Abstract

In *Schizosaccharomyces pombe* the repair of apurinic/apyrimidinic (AP) sites is mainly initiated by AP lyase activity of DNA glycosylase Nth1p. In contrast, the major AP endonuclease Apn2p functions by removing 3′-α,β-unsaturated aldehyde ends induced by Nth1p, rather than by incising the AP sites. *S. pombe* possesses other minor AP endonuclease activities derived from Apn1p and Uve1p. In this study, we investigated the function of these two enzymes in base excision repair (BER) for methyl methanesulfonate (MMS) damage using the *nth1* and *apn2* mutants. Deletion of *apn1* or *uve1* from *nth1*Δ cells did not affect sensitivity to MMS. Exogenous expression of Apn1p failed to suppress the MMS sensitivity of *nth1*Δ cells. Although Apn1p and Uve1p incised the oligonucleotide containing an AP site analogue, these enzymes could not initiate repair of the AP sites *in vivo*. Despite this, expression of Apn1p partially restored the MMS sensitivity of *apn2*Δ cells, indicating that the enzyme functions as a 3′-phosphodiesterase to remove 3′-blocked ends. Localization of Apn1p in the nucleus and cytoplasm hints at an additional function of the enzyme other than nuclear DNA repair. Heterologous expression of *Saccharomyces cerevisiae* homologue of Apn1p completely restored the MMS resistance of the *nth1*Δ and *apn2*Δ cells. This result confirms a difference in the major pathway for processing the AP site between *S. pombe* and *S. cerevisiae* cells.

Keywords: DNA repair; Base excision repair; *Schizosaccharomyces pombe*; AP sites; AP endonuclease; *apn1*; *apn2*; *uve1*; *nth1*

In all organisms apurinic/apyrimidinic (AP) sites arise spontaneously by hydrolysis of the *N*-glycosylic bond, and are introduced by the action of monofunctional DNA glycosylases removing damaged, modified, or mismatched bases from DNA [1]. AP sites are also induced directly by ionized radiation and reactive oxygen species [1]. Base excision repair (BER) is the major DNA repair pathway for repair of AP sites, and is initiated by cleavage of the phosphodiester bond either by AP endonuclease or the AP lyase activity of bifunctional DNA glycosylase (for review, see [2,3]). AP endonuclease hydrolyzes a phosphodiether bond 5′ to the AP site to leave a 3-hydroxyl group and a 5′-deoxyribose phosphate (5′-dRp). Next a DNA polymerase creates a flap structure with the 5′-dRp end, which is then removed by the flap endonuclease. AP lyase cleaves 3′ to the AP site, leaving a 3′-α,β-unsaturated aldehyde and a 5′-phosphate. An AP endonuclease with 3′-phosphodiesterase activity is also employed to repair 3′-α,β-unsaturated aldehyde blocks, and then a short gap is filled. The BER process is accomplished by strand joining with DNA ligase.

Schizosaccharomyces pombe Nthp1 is a bifunctional DNA glycosylase, which removes oxidized pyrimidine and incises the AP site, leaving a 3′-blocked end [4]. Nth1p is responsible for the majority of activity for thymine glycol and AP site incision in the absence of metal ions [5]. Deletion of *nth1* exhibits hypersensitivity to methyl methanesulfonate (MMS) [5–7]. Apn2p, the major AP endonuclease in *S. pombe*, also greatly contributes to the repair of MMS damage [8]. Deletion of *nth1* from an

☆ *Abbreviations:* AP, apurinic/apyrimidinic; BER, base excision repair; GFP, green fluorescent protein; MMS, methyl methanesulfonate; THF, tetrahydrofurane; 5′-dRp, 5′-deoxyribose phosphate.

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apn2 mutant resulted in tolerance to MMS damage, indicating that Nth1p-induced 3′-blocks are responsible for MMS sensitivity in *apn2* mutants [5,7]. Overexpression of Apn2p in *nth1* mutants failed to suppress MMS sensitivity [5]. These results indicate that Nth1p, not Apn2p, primarily incises AP sites and that the resultant 3′-blocks are removed by the 3′-phosphodiesterase activity of Apn2p (Nth1p-dependent pathway) (Fig. 1).

Schizosaccharomyces pombe Apn2p shares homology with *Escherichia coli* exonuclease III, *Saccharomyces cerevisiae* Apn2p, and human APE2 [9–11]. *S. pombe* possesses another type of AP endonuclease Apn1p, a homologue of *E. coli* endonuclease IV [12,13]. The Apn1p homologue of *S. cerevisiae* contributes the major activity of AP endonuclease and functions in the initial step of BER for AP sites in yeast [12,14]. However, a *S. pombe apn1* mutant was insensitive to MMS damage [8,13]. The function of Apn1 *in vivo* has so far been scarcely characterized, because of its low activity in *S. pombe* cells. Uve1p, which is primarily a UV-photoproduct specific endonuclease of *S. pombe*, could recognize non-UV-induced DNA damage, including the AP site *in vitro*, and hydrolyze immediately 5′ to the damage [15]. The enzyme functions mainly in the initial step of an alternative excision repair pathway for the removal of DNA damage caused by exposure to UV light [16,17]. *S. cerevisiae* has no reported homologue of Uve1p. *Neurospora crassa* Uve1p has been shown to complement bacterial BER mutants (*xth*$^{-}$ *nfo*$^{-}$) with respect to sensitivity to MMS and an oxidative agent, *t*-butyl hydroperoxide [18].

In this study, we investigated the function of *S. pombe* Apn1p and Uve1p in processing AP sites using a genetic approach. Although Apn1p and Uve1p showed the AP endonuclease activity *in vitro*, the enzymes could not

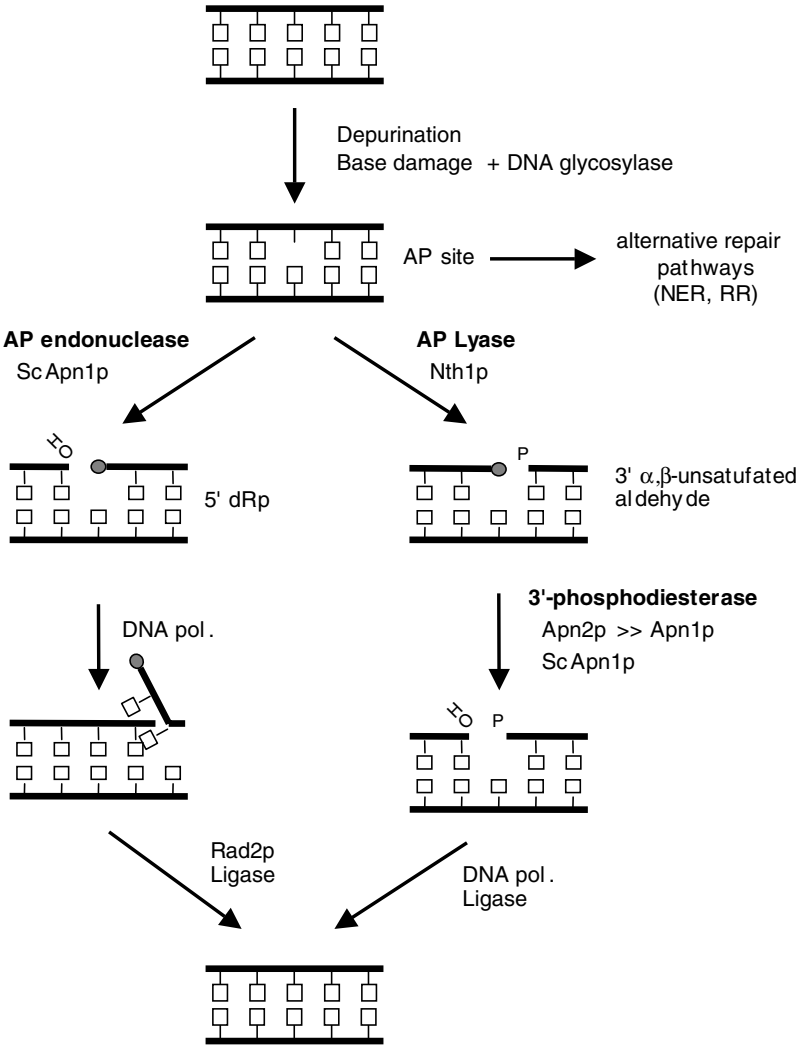

Fig. 1. BER pathway for processing of the AP site in *S. pombe*. In the Nth1p-dependent pathway the AP site repair is initiated by incision of Nth1p leaving 3′-α,β-unsaturated aldehyde termini. Apn2p functions primarily in the removal of the 3′-blocked termini, and poorly in the incision of AP sites. The repair will be completed by filling single-nucleotide gaps followed by sealing of the phosphodiester bond. Apn1p is a back-up enzyme for 3′-phosphodiesterase activity of Apn2p. A part of the AP sites could be repaired by nucleotide excision repair and recombination repair. *S. cerevisiae* Apn1, but not *S. pombe* Apn1p, Apn2p, and Uve1p, could incise the AP site sites to leave 5′-dRp ends, which will be removed by Rad2p after DNA synthesis. NER, nucleotide excision repair; RR, recombination repair; *Sc*, *S. cerevisiae*.