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Resolution of a complex crisis at DNA 3'-termini

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Abstract

Ribonucleotides that are mis-incorporated into DNA during replication are removed by Topoisomerase 1, which generates 3' terminal adducts that are not amenable to DNA repair and thus compromise genome stability. A recent report by Niu and colleagues reveals that Apn2/APE2 resolves such blocked 3'-termini to suppress Top1-induced mutations at rNMP sites within the genome.

Ribonucleotide monophosphates (rNMPs) that are mis-incorporated into the genome during DNA replication give rise to DNA replication stress and mutations that are a source of genome instability¹. Replicative DNA polymerases insert approximately one rNMP for every 6,500 deoxyribonucleotides synthesized in budding yeast, and more than 1,000,000 rNMPs may be incorporated during replication of the mouse genome². Widespread, non-randomly distributed rNMPs have been mapped in the *S. cerevisiae* genome by recently developed genome-wide ribose-seq approaches³. Genomic rNMPs are removed via RNase H2-mediated excision repair or by Topoisomerase 1 (Top1)-catalyzed cleavage^{2,4}. The latter process generates a single-strand break (SSB) or nick with a 5'-hydroxyl group and complex, 3'-blocking termini such as 2',3'-cyclic phosphate or a Top1 cleavage complex (Top1cc) (Fig. 1)⁵. Without appropriate end resection and processing, these Top1 products are mutagenic because the 'blocked' 3' ends impair DNA repair^{1,4}.

Srs2 helicase and exonuclease 1 (Exo1) mediate 5'-end resolution of Top1-generated SSBs to generate a short gap that can be faithfully repaired⁶, but it was not known how the 3' complex termini are processed. Using a combination of genetic and biochemical approaches, Li *et al.* now reveal that Apn2/APE2 (AP endonuclease 2, also known as APEX2) resolves blocked 3' ends of Top1-induced SSBs/nicks to avoid introducing mutations at rNMP sites in the genome⁷. A systematic survey of several candidate 3'-end processing enzymes in budding yeast identified Apn2/APE2 as the key nuclease that resolves a wide range of blocked 3' ends, including terminal 2', 3'-cyclic phosphate and monophosphate. Apn2/APE2 also processes Top1cc intermediates *in vitro* and suppresses Top1-induced mutagenesis *in vivo*. These findings shed new light on the novel mechanism of Apn2/APE2 function in the maintenance of genome integrity.

Apn2/APE2 is an evolutionarily conserved protein with multiple nuclease activities. Apn2 was identified in budding yeast as the second homolog of *E. coli* Exo III and human APE1/Ref1, and genetic evidence established its importance in repair of DNA alkylation damage

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and apurinic/aprimidinic (AP) sites⁸. Subsequently, human APE2 was identified as a base excision repair (BER) protein that primarily localizes to the nucleus but also found in mitochondria^{9,10}. *Xenopus* APE2 is required for activation of the ATR-Chk1 DNA damage response (DDR) pathway following oxidative stress¹¹.

Apn2/APE2 contains an N-terminal EEP (endonuclease/exonuclease/phosphatase) domain, a middle PIP box (PCNA interacting protein) motif, and a C-terminal Zf-GRF (zinc finger domain with conserved GRxF motif) domain^{10,12}. Biochemical characterization has demonstrated that Apn2/APE2 possesses strong 3'–5' exonuclease and 3'-phosphodiesterase activity but weak AP endonuclease activity^{13–15}. PCNA interactions via the PIP box and Zf-GRF motifs stimulate nuclease activity in yeast, *Xenopus*, and humans^{12, 15–17}, where Apn2/APE2 plays multiple critical roles in DNA repair.

The study by Li *et al.* illuminates new aspects of Apn2/APE2 function in genome integrity maintenance. While prior biochemical analyses identified AP sites within double-strand DNA (dsDNA), 3'-recessed dsDNA with a 3'-OH, 3'-phosphoglycolate (3'-PG), or 3'-mismatched nucleotide, as well as nicked and gapped dsDNA structures as Apn2/APE2 substrates^{12–14}, the present work reveals additional substrates associated with rNMP removal⁷, including ribonucleotides with a 2',3'-cyclic phosphate or monophosphate, and 3'-phosphotyrosine-DNA conjugates characteristic of Top1cc intermediates. The 3' termini generated by Top1-mediated rNMP cleavage block the 3' exonuclease activity of Pol delta and must be resolved to permit DNA repair. Although Apn2/APE2 resects only two nucleotides at 2',3'-cyclic phosphate-terminated ends *in vitro*, PCNA stimulates exonuclease-mediated resection of ~8–10 nt in the 3'–5' direction. In the presence or absence of PCNA, ~2–11 nt are resected from ends bearing Top1cc adducts. Importantly, Apn2/APE2 resection produces 3'-recessed DNA with 3'-OH that is suitable for Pol delta-catalyzed extension and synthesis. These products are different to those observed when Top1cc are removed by Tdp1, which generates a terminal 3'-phosphate that is further processed by Tpp1 to generate 3'-OH. Thus Apn2/APE2 removes mis-incorporated rNMPs via a distinct mechanism.

While the process of double-strand break repair has been extensively investigated, much less is known about the mechanisms underlying processing and repair of SSBs. The demonstration that 'blocked' 3' ends of Top1-induced SSB are resected by Apn2/APE2 is reminiscent of a recently proposed 3'–5' SSB end-resection mechanism for APE2 in genome integrity maintenance¹⁸. Similar to Srs2 deletion, Apn2 deletion is lethal in *pol2-M644G rnh201* yeast cells that lack RNase H2 and harbor a Pol epsilon mutation^{6,7}. However, Apn2/APE2 processes both 3'-recessed and nicked dsDNA bearing 'blocked' 3' ends *in vitro*, suggesting that a 5' gap may not be required for Apn2/APE2 to resolve these 3' ends⁷. It is not yet known whether Srs2-Exo1 mediated 5–3' end resection and Apn2/APE2-catalyzed 3'–5' end resection are dependent on each other *in vivo*. Future investigation is needed to directly address this question.

How does the role of Apn2/APE2 in maintaining genome integrity contribute to cell physiology? Prior studies elucidated functions of Apn2/APE2 in BER and SSB repair pathways, and Li *et al.* now establish the importance of Apn2/APE2 in rNMP repair and

preventing Top1-induced mutations in budding yeast. It will be important to determine whether this novel function of Apn2/APE2 is conserved in other organisms, including humans. In this regard, recent genetic analyses have revealed that APE2 is a synthetic lethal target of BRCA2 in mammalian cells¹⁹ and APE2 has also been identified as an important regulator of epigenome and genome integrity in *Arabidopsis thaliana*²⁰. Further investigation is necessary to determine the potential relevance of Apn2/APE2 function to human disease pathology.

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Fig 1. Apn2/APE2 suppresses Top1-induced mutations at rNMP sites.

Cleavage of genomic ribonucleotides by Top1 produces a nick with a 2',3'-cyclic phosphate or monophosphate at the 3' terminus. The 5'-terminus is resected by Srs2-Exo1 in the 5'–3' direction, while the 3' end is progressively digested by Apn2/APE2 in the 3'–5' direction. The gap generated by 5' and 3' end processing is subsequently filled and ligated, leading to error-free repair. It is not known whether the 5' - and 3'-end processing is sequential or occurs simultaneously. Additional cleavage by Top1 can generate a Top1 cleavage complex (Top1cc) that is removed by either Apn2/APE2 or Tdp1-Tpp1. In the absence of processing, DNA ends cleaved and ligated by Top1 generate a genomic deletion.