APE1 Follows the Induced Space-Filling Mechanism to Distinguish and Processing Various DNA Substrates

Combining APE1-DNA complex structures and biochemical assays, we established a space-filling model to depict how APE1 distinguishes various substrates and to reveal the structural basis of the drug-resistant mechanism associated with APE1.

APE1 is a DNA-repair enzyme with both endonuclease and exonuclease activity. As an endonuclease, the apurinic/apyrimidinic site (AP site)-specific cleavage of APE1 is an essential process to repair oxidized nucleotides in the base excision repair (BER) path. As an exonuclease, APE1 digests various deoxynucleotides at the 3′- termini of matched or 1 nt-mismatched duplex DNA without base preference. The non-specific exonuclease activity of APE1 leads to nucleoside analogue-typed anti-cancer drug resistance. Excision of the 3′-end-matched base pair by APE1 in various structural duplex DNA, such as gapped, nicked and recessed dsDNA, is vital for a series of DNA processing paths, including nucleotide incision repair, Trinucleotide repeat expansion-related BER, single-strand breaks and apoptosis. Unlike the well studied endonuclease activity, the lack of structural information leaves the molecular details of the base and structural preference of APE1 exonuclease activity still missing. In this study, we determined two terminal-binding structures of the APE1-dsDNA complex and demonstrated an induced space-filling model to reveal the structural basis of its exonuclease activity. The X-ray diffraction data sets were collected at beamlines **TLS 13B1**, **TLS 15A1**, and **TPS 05A** of the NSRRC.[1]

After DNA binding, Arg176 and Met269 of APE1 are induced to form a bridge-like structure (RM bridge) occupying the active site and creating a narrow channel (**Figs. 1(a-c)**). A RM bridge separates the active site into the catalytic site and a product pocket in which the unique structure conducts the different substrate specificity of APE1 endo- and exo-nuclease activity. In the endonuclease manner, APE1 is an AP site-specific nuclease because the steric hindrance from the downstream nucleotide and narrow product pocket makes a normal nucleotide that cannot be accommodated (**Fig. 1(d)**). In the

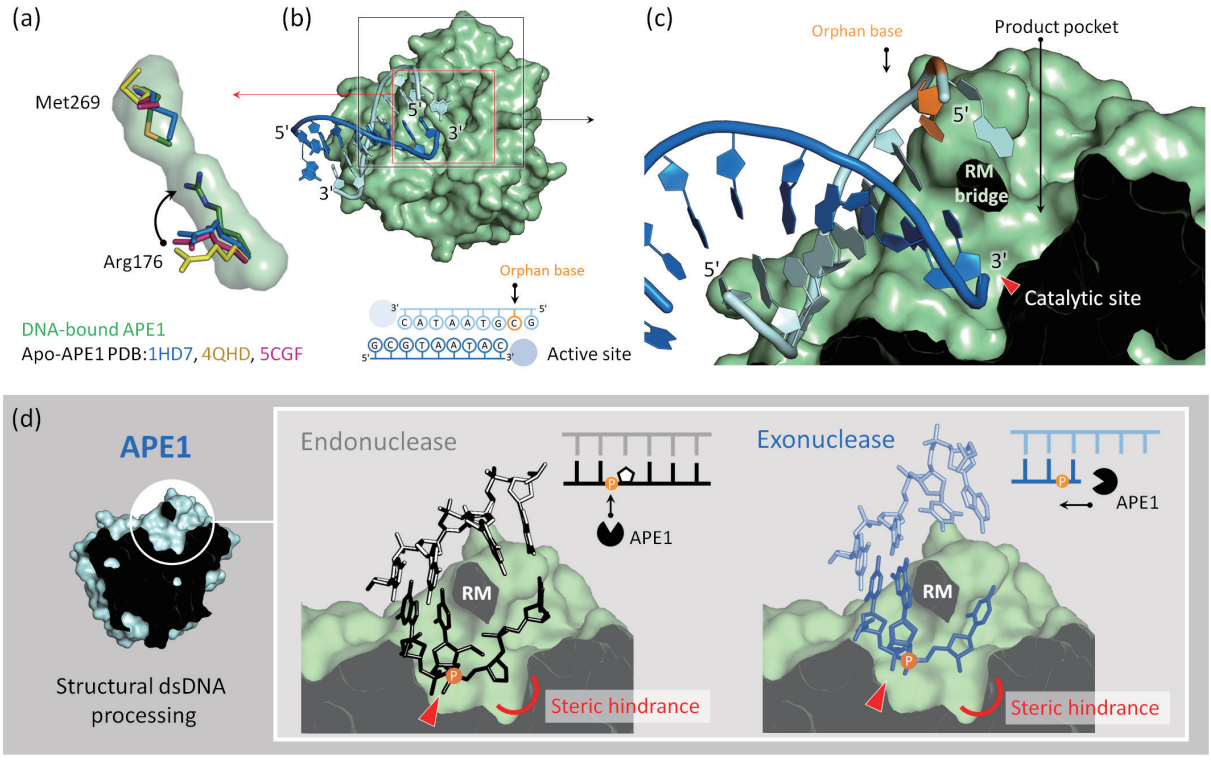

Fig. 1: (a,b) APE1-recessed dsDNA product complex structures and conformational changes of the RM bridge. (c) The RM bridge occupies the space of the active site and separates the active site into the catalytic site and a product pocket. (d) A magnified view of the active site when APE1 works as an endonuclease or exonuclease. The structural comparison reveals the structural basis of the base preference of APE1. [Reproduced from Ref. 1 and 2]

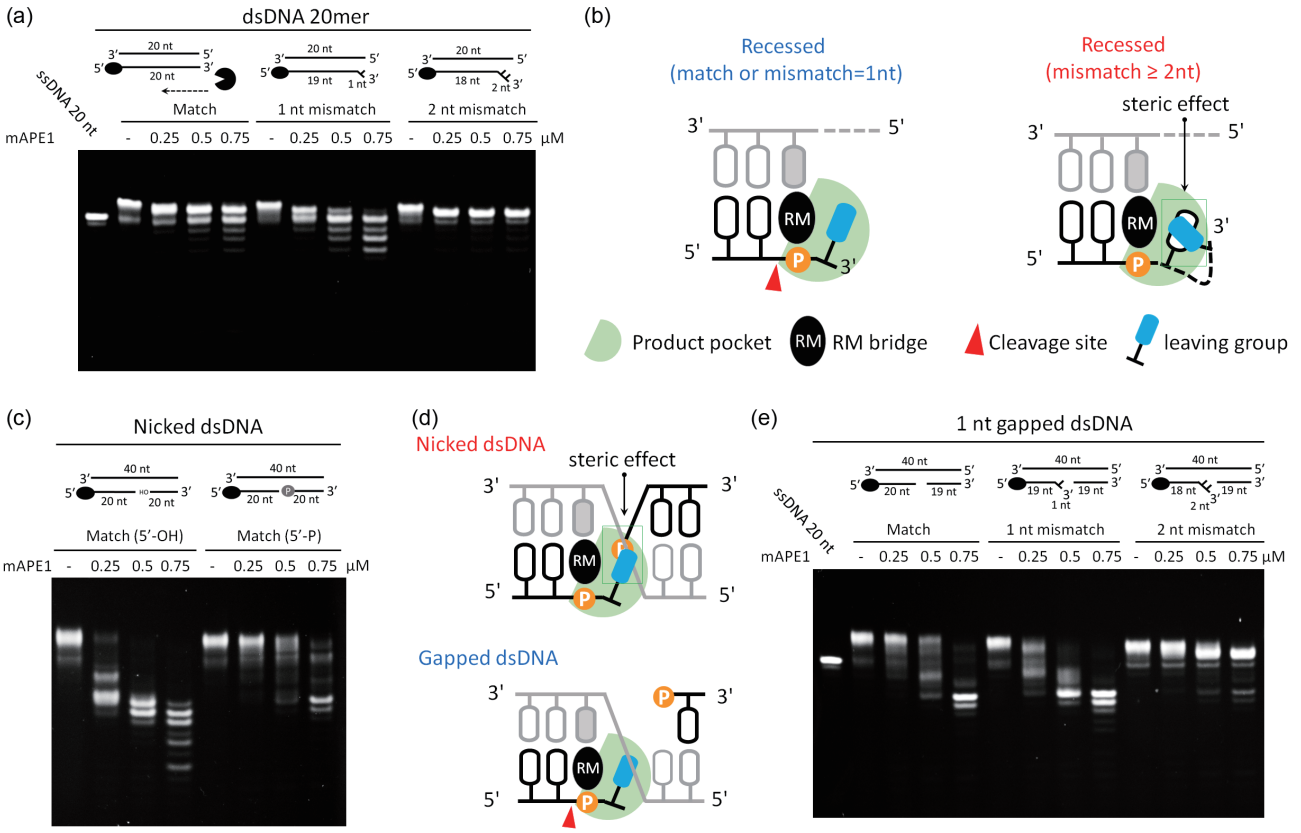

Fig. 2: (a) Exonuclease activity of mAPE1 in digesting dsDNA with blunt-end, 1-nt mismatch and 2-nt mismatch. dsDNA with 2-nt mismatch is a poor substrate of APE1 exonuclease. (b) An induced space-filling model depicts why dsDNA with 2-nt mismatch is a poor substrate. (c) Exonuclease activity of mAPE1 in digesting nicked dsDNA with or without flank 5′-phosphate. (d) The induced space-filling model depicts how the APE1 exonuclease activity is blocked by nicked dsDNA with flank 5′-phosphate, and why APE1 can digest gapped dsDNA. (e) Nuclease activity of APE1 on gapped dsDNA. [Reproduced from Ref. 1 and 2]

exonuclease manner, the last nucleotide base can rotate and be placed into another region of the product pocket (**Fig. 1(d)**). In addition, the product pocket is composed of hydrophobic residues that cannot specifically interact with nitrogenous bases with hydrogen bonds. These two structural features make APE1 exonuclease without a base preference.

This induced space-filling mechanism also clarifies the selection mechanism for the structural preference of APE1 exonuclease on dsDNA that is critical to identify the role of APE1 on various DNA repair paths. First, APE1 prefers to digest dsDNA with blunt-end and 1 nt-mismatch rather than the 2 nt-mismatch (**Fig. 2(a)**), because the narrow product pocket with steric hindrance cannot accommodate two nucleotides; APE1 can thus difficultly digest substrates with more than 2 nt-mismatched bases (**Fig. 2(b)**). In contrast, the nicked dsDNA with flank 5′-phosphate is an unfavourable substrate for the APE1 exonucleolytic cleavage (**Fig. 2(c)**). The steric hindrance between the base region of 3′-terminal deoxyribonucleotide and the downstream 5′-phosphate group leads to an unfavourable cleavage of 5′-phosphate nicked dsDNA (**Fig. 2(d)**). This hypothesis is proved with nuclease activity assays in vitro. With the substrates without steric hindrance, such as nicked dsDNA with the flank 5′-hydroxy group and gapped dsDNA, the exonuclease activity of APE1 is no longer restricted (**Figs. 2(c) and 2(e)**).

In summary, the DNA binding-induced RM bridge fills the space of the APE1 active site to create a narrow channel-liked structure. The narrow hydrophobic product pocket enables the APE1 with different base selection mechanisms in endo- and exo-nuclease activity. When APE1 works as an endonuclease, it targets only the AP site in the middle of dsDNA. When APE1 works as an exonuclease, it removes any nucleotide at the 3′-terminal of duplex DNA without preference. Additionally, the steric hindrance of the product pocket weeds out the dsDNA with longer 3′-overhang and nicked dsDNA with flank 5′-phosphate to construct the structural preference of APE1. Our unprecedented induced space-filling model clarifies the base and structural selection mechanisms of APE1 in processing dsDNA and paves the way to understand the cellular functions and drug resistance associated with APE1.[1,2] (Reported by Tung-Chang Liu and Jung-Yu Liu, , National Yang Ming Chiao Tung University)

This report features the work of Yu-Yuan Hsiao and his collaborators published in Nat Commun. ***12****, 601 (2021).*

TLS 13B1 Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography
TPS 05A Protein Microcrystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

References

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Poa1p Macro Domain Structure: Deciphering a Non-Canonical 3"-*O*AADPR Deacetylase

In this report, the undefined enzymatic activity and substrate specificity of the Poa1p macro domain were revealed on deciphering the crystal structures in a combination of biochemical approaches and guided a study of biological effects of the OAADPR metabolism in epigenetics.

O-acetyl-ADP-ribose (*O*AADPR) is a fascinating signaling molecule that has been implicated in the regulation of numerous cellular processes, including the formation of silent information regulator complexes and gene silencing. This molecule was first identified from the conserved NAD-dependent histone/protein deacetylase reaction catalyzed by sirtuins. In cells, the ratio of 2"- and 3"-*O*AADPR exists in equilibrium as 48:52 at slightly alkaline pH through a non-enzymatic intermolecular transesterification.[1]

There is accumulating evidence that a group of macro domain proteins efficiently catalyzes the deacetylation of 2"-*O*AADPR, including MacroD-like proteins (human MacroD1, human MacroD2, *E. coli* Ymdb, *O. iheyensis* MacroD), the sirtuin-linked macro domain SAV0325 from *S. aureus*, and the human TARG1-like macro domain C6orf130.[2] The catalysis ability of macro domains stems from their physical or genetic link with sirtuins, thus revealing the functional connections with sirtuins and a novel aspect of *O*AADPR metabolism.[3] To date, only 2"-*O*AADPR deacetylases but no 3"-*O*AADPR deacetylase was reported.

S. cerevisiae contains sirtuins as a major NAD-consuming family but lacks any poly(ADP-ribose) polymerase (PARP) homologues, which indicates that sirtuin reaction product *O*AADPR might hold the most potential physiological roles in yeast. Qualitative analysis of yeast cell extracts revealed at least three distinct activities contributing to the metabolism of *O*AADPR *in vivo*, but only Nudix hydrolase Ysa1 was reported to hydrolyze *O*AADPR/ADPR to AMP and ribose phosphate or acetyl-ribose phosphate, thereby lowering the cellular *O*AADPR/ADPR levels.[4] Protein identities of the unknown deacetylase and acetyl transferase remain obscure.[2,4] Poa1p is a unique fungal-type macro domain protein that shares little sequence homology with other macro domains. To explore the function of the uncharacterized macro domain, a research team led by Chun-Hua Hsu (National Taiwan University) determined the structures of *apo*, ADPR-bound Poa1p, and its mutants. The diffraction data were collected at **TPS 05A**, **TLS 15A1** and **TLS 13B1**.[5]

Our biochemical data reveals that, within two macro domains from *S. cerevisiae*, only Poa1p carried a robust catalytic activity toward deacetylation of *O*AADPR, yielding a significant reaction product, ADPR. To elucidate enzymatic properties of Poa1p at a molecular level, the crystal structure of ADPR-bound Poa1p was determined (**Fig. 1(a)**). The structure of Poa1p-ADPR complex, at resolution 1.78 Å, adopts a three-layered α/β/α sandwich fold with the ADPR molecule accommodated within the central crevice of Poa1p. This condition strongly indicates a typical macro domain fold for ADPR binding. Having structurally characterized the Poa1p macro domain, the authors then investigated the substrate specificity of Poa1p for *O*AADPR deacetylation. The RP-HPLC and ^{18}O-labeling results revealed that Poa1p can hydrolyze both 3"- and 1"-*O*AADPR isomers, and preferentially facilitate 3"-*O*AADPR cleavage at neutral pH. To decipher the molecular mechanism of this finding, interactions between Poa1p and ADPR were examined (**Fig. 1(b)**, see next page).