APE2 Zf-GRF facilitates 3'–5' resection of DNA damage following oxidative stress

Bret D. Wallace1,2, Zachary Berman1–3, Geoffrey A. Mueller1, Yunfeng Lin1, Timothy Chang1, Sara N. Andres1, Jessica L. Wojtaszek2, Eugene F. DeRose3, C. Denise Appel1, Robert E. London4, Shan Yan2,5, and R. Scott Williams1,2

*Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC 27709 and 3Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC 28223

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The Xenopus laevis APE2 (apurinic/apyrimidinic endonuclease 2) nuclease participates in 3'–5' nucleolytic resection of oxidative DNA damage and activation of the ATR-Chk1 DNA damage response (DDR) pathway via ill-defined mechanisms. Here we report that APE2 resection activity is regulated by DNA interactions in its Zf-GRF domain, a region sharing high homology with DDR proteins Topoisomerase 3α (TOP3α) and NEIL3 (Nei-like DNA glycosylase 3), as well as transcription and RNA regulatory proteins, such as TTF2 (transcription termination factor 2), TFIIIS, and RPB9. Biochemical and NMR results establish the nucleic acid-binding activity of the Zf-GRF domain. Moreover, an APE2 Zf-GRF X-ray structure and small-angle X-ray scattering analyses show that the Zf-GRF fold is typified by a crescent-shaped ssDNA binding claw that is flexibly appended to an APE2 endonuclease/exonuclease/phosphatase (EEP) catalytic core. Structure-guided Zf-GRF mutations impact APE2 DNA binding and 3'–5' exonuclease processing, and also prevent efficient APE2-dependent RPA recruitment to damaged chromatin and activation of the ATR-Chk1 DDR pathway in response to oxidative stress in Xenopus egg extracts. Collectively, our data unveil the APE2 Zf-GRF domain as a nucleic acid interaction module in the regulation of a key single-strand break resection function of APE2, and also reveal topologic similarity of the Zf-GRF to the zinc ribbon domains of TFIIIS and RPB9.

Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses (1, 2). This presents a major challenge to genomic stability (3–6) and is implicated in the pathogenesis of multiple human diseases, including cancer and neurodegenerative disorders (7, 8). Abundant DNA damage from oxidative stress constitutes ~10% of all DNA lesions and includes oxidative DNA damage, such as base damage, sugar moieties damage, apurinic/apyrimidinic (AP) sites, DNA single-strand breaks (SSBs), and double-strand breaks (DSBs) (2, 6). Repair of oxidative lesions involves primarily the base excision repair (BER) pathway (9, 10). Although the details of DNA damage recognition and corrective processes are well documented, the molecular mechanisms for activation of oxidative DNA damage and DNA replication stress (3, 5, 11). Whereas ATM is activated by autophosphorylation and dimer dissociation in response to DSBs (12, 13), ATR is activated by primed single-stranded DNA (ssDNA) from the functional uncoupling of minichromosome maintenance (MCM) helicase and DNA polymerase activities at stalled replication forks or DNA end resection of DNA strand breaks in the 5'–3' direction (3, 14). ATR activation requires several mediator proteins, such as ATRIP (ATR-interaction protein), TopBP1, and the 9–1–1 (Rad9–Rad1–Hus1) complex (15–18). Activated ATR phosphorylates a variety of substrates, including Chk1 (19). Chk1 phosphorylation serves as an indicator of ATR activation, and activated Chk1 kinase phosphorylates its own substrates, such as Cdc25, to arrest cell cycle progression (20, 21). We recently established that the ATR-Chk1 checkpoint is triggered by oxidative DNA damage in Xenopus egg extracts (2, 22). Here, checkpoint activation critically requires the APE2 (apurinic/apyrimidinic endonuclease 2) 3'–5' resection nuclease. Our understanding of APE2 molecular functions in this process remains poorly delineated, however.

APE2 (also termed APEX2, or Apn2 in yeast) is a minor AP endonuclease (23–25) that, compared with APE1, harbors weak AP endonuclease activity but robust proliferating cell nuclear antigen (PCNA)-stimulated 3'–5' exonuclease and 3'–phosphodiesterase activities (24, 26, 27). APE2-null mice exhibit growth retardation and dyslymphopoiesis accompanied by G2/M arrest, suggesting its importance for proper cell cycle progression (28). In mammalian cells, APE2 is also necessary for normal B-cell development and recovery from chemotherapy drug-induced DNA damage (29). It was recently proposed that differential expression of APE2 in germinal centers promotes error-prone repair and mutations during somatic hypermutation (30). Importantly, APE2 is a key player in the PCNA-dependent repair of hydrogen peroxide-induced oxidative DNA damage (31–33).

Significance

Zf-GRF domains are found in more than 100 eukaryotic architectures, including key proteins modulating DNA damage response and transcription. We establish the apurinic/apyrimidinic endonuclease 2 (APE2) Zf-GRF domain as a prototypical member of the Zf-GRF class of nucleic acid-binding modules, and through structural analysis reveal that the APE2 protein is composed of a compacted three-stranded β-sheet and a CHC Zn2+–binding site, harboring structure-specific ssDNA-binding activity. Notably, the ssDNA-binding region of APE2 Zf-GRF is required for the 3'-5' end resection of oxidative DNA damage and activation of the ATR-Chk1 DNA damage response pathway following oxidative stress. This distinct regulatory mechanism of APE2 exonuclease activity by ssDNA binding via Zf-GRF may extend to other Zf-GRF-containing proteins.


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B.D.W. and Z.B. contributed equally to this work.

To whom correspondence may be addressed. Email: shan.yan@unc.edu or williamsr@niehs.nih.gov.

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Although APE2 enzymatic activities and associated functions in DNA repair and oxidative stress have been characterized in different experimental systems, including *Arabidopsis thaliana*, *Trypanosoma cruzi*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Homo sapiens* (33–39), a detailed mechanistic understanding of conserved APE2 functions is lacking. In particular, compared with other EEP (endonuclease/exonuclease/phosphatase) nuclease family members, APE2 is distinguished by the presence of a highly conserved zinc finger motif containing GRxF residues (hereinafter designated Zf-GRF) of unknown function. Herein we provide evidence that the APE2 C-terminal Zf-GRF preferentially associates with ssDNA and plays a central, unexpected role in regulating the 3′→5′ exonuclease activity. Using combined structural, biochemical, and functional approaches, we further establish that the Zf-GRF domain is crucial for APE2’s role in activating the ATR-Chk1 DDR pathway following oxidative stress.

**Results**

**Mapping APE2 Functional Domains.** The molecular underpinnings for APE2 resection activities are ill-defined. To delineate APE2 functional domains, we coupled predictions of protein order/disorder to limited trypsin proteolysis and small-angle X-ray scattering (SAXS) studies of APE2. We purified full-length recombinant *Xenopus laevis* APE2 (APE2FL, residues 1–517) and subjected it to limited chymotrypsin proteolysis that identified stable regions of APE2 encompassing the EEP catalytic domain core (residues 1–336) and a folded domain bounding a predicted “Zf-GRF” region (amino acids 445–517). Proteolysis results closely correlate with predicted regions of protein disorder derived from D²P² (40) (Fig. 1A and B). Additional observations from SAXS (SI Appendix, Results and Discussion and Figs. S1–S4) are also consistent with the EEP nuclease core of APE2 being flexibly tethered to a structured C-terminal Zf-GRF domain via a flexible region that includes a PCNA-interacting protein (PIP) box motif.

**Molecular Architecture of the Zf-GRF.** The C-terminal Zf-GRF is a defining feature of APE2 that distinguishes it from other EEP-containing enzymes (SI Appendix, Fig. S5A). Zf-GRF domains are 45- to 50-residue domains widely distributed throughout Eukarya that are found in >100 unique domain architectures (pfam.xfam.org/family/zf-GRF), including several human proteins involved in DDR, transcriptional regulation, and RNA processing (SI Appendix, Fig. S5B). The function of Zf-GRF domains in APE2 and other proteins remains undefined, however. To characterize the Zf-GRF molecular structure, we first crystallized and determined the crystal structure of the APE2 Zn-GRF domain. The APE2 domain structure is composed of an N-terminal helix (α1) connected by a polyproline helix to three antiparallel β-sheets (β1–β3) containing the Zn coordination site and the GRF motif. The model phased anomalous difference Fourier map is indicated in red (5.0σ), and the final Zn-GRF map is in blue (2.0σ). The polyproline helix of the Zf-GRF structure overlaid with the collagen crystal structure showing high structural similarity. (B) Electrostatic surface representation of the front and side views of the Zf-GRF domain. The electrostatic potential was calculated in APBS and rendered in PYMOL to display ±3 kT/e. An electropositive patch that interacts with a bound sulfate molecule is shown as yellow and red sticks. Key conserved lysine and arginine residues are labeled.
the X-ray structure of a fragment of APE2 encompassing the chymotrypsin stable Zf-GRF core (Fig. 1B), plus an N-terminal extension to this domain that is conserved among APE2 homologs (XlApe2 amino acids 436–517) (Fig. 2A). Consistent with a predicted Zn$^{2+}$-binding fold, we detected a strong anomalous signal at the Zn$^{2+}$ absorption edge in our crystals. Accordingly, we solved the structure using Zn$^{2+}$ single-wavelength anomalous dispersion (SAD) phasing (to 2.60 Å; SI Appendix, Table S1).

Overall, the Zf-GRF comprises a three-stranded anti-parallel β-sheet (β1–β3) that folds into a crescent-shaped claw-like structure. A single bound Zn$^{2+}$ ion plays a central structural role in this domain, and is coordinated with tetrahedral geometry by a His of this motif with a Cys residue (CCCC-coordination). The first two Zn$^{2+}$ ligands (C463 and H466) are found in a loop preceding β1, whereas the second half of the motif (C489 and C503) maps to the β2-β3 connecting loop. N-terminal to the Zf-GRF, APE2 homologs also contain a proline-rich region. Intriguingly, five consecutive prolines in APE2 adopt a collagen-like helical conformation and bridge the Zf-GRF to an extended α-helix, α1 (Fig. 2A, Inset). These three elements the N-terminal α helix, polyproline helix, and Zf-GRF—combine to form an elongated crutch-shaped molecule in the dsDNA. Similarly, Chemical shift mapping of the Zf-GRF–dsDNA interaction. Mapping of chemical shifts onto the Zf-GRF crystal structure. Regions of high perturbation on incubation with ssDNA (Fig. 3 and SI Appendix). The concave surface of the Zf-GRF likely embraces the nucleic acid (Fig. 3, Left). In contrast, the backbone (convex surface) of Zf-GRF is relatively unresponsive to ligand (Fig. 3, Right). Three ssDNA-binding clusters, denoted DNA-binding regions 1–3 (DBR 1–3), are identified with significant chemical shift perturbation on incubation with ssDNA (SI Appendix, Fig. S7A and B). Intriguingly, these regions include the conserved Zf-GRF basic residue clusters R473/K476/K477, which bind sulfate in our crystals, and R502 in the β2–β3 connecting loop, which we predict will interact with the DNA phosphate backbone. In addition, a clustering of conserved aromatic residues from β1 (V475), β2 (F486 and V488), and β3 (F506 and W508) display moderate ssDNA-induced chemical shift perturbation of the backbone amid. Together, these residues form a contiguous hydrophobic surface that may serve to base stack with the exposed ssDNA nucleobases (Fig. 3).

**Zf-GRF Is a Structure-Specific DNA-Binding Domain.** The APE2 Zf-GRF bears a distinctive electrophoretic groove on its concave surface (Fig. 2B). We observed a bound sulfate (SO$_4^{2−}$) molecule salt-bridged to the conserved basic residues K476 and R473. With K477, R484, R491, and R502, these positively charged residues combine and form a contiguous extended basic surface that appears appropriate for binding nucleic acid (Fig. 2B). To test this hypothesis using electrophoretic mobility shift assays (EMSA), we evaluated the ability of maltose binding protein (MBP)–Zf-GRF fusion proteins to interact with variable DNA secondary structures, including ssDNA, dsDNA, DNA bubbles, 5′- and 3′-flaps, and forked DNA duplexes (SI Appendix, Fig. S6A). Strikingly, the Zf-GRF displayed high-affinity DNA binding to substrates containing single-stranded regions. Substrates with considerable ssDNA character bound best, in the order ssDNA > fork > 3′ and 5′ flaps > dsDNA. Similarly, quantification of DNA-binding affinities using fluorescence polarization equilibrium binding analysis of FAM-labeled DNA substrates showed that Zf-GRF binds ssDNA (180 ± 40 nM), or a 3′-recessed substrate (560 ± 74 nM, a presumably relevant substrate for APE2), in the nanomolar range, but does not interact appreciably with dsDNA (SI Appendix, Fig. S6B).

To more directly evaluate DNA binding in solution, we examined interactions of the Zf-GRF with ssDNA by chemical shift perturbation of 2$^3$N-labeled Zf-GRF protein (amino acids 460–511). Overall, we observed a pronounced chemical shift response of Zf-GRF to incubation with a 10-nt ssDNA target (Fig. 3 and SI Appendix, Fig. S7A). This analysis indicates that the concave surface of the Zf-GRF likely embraces the nucleic acid (Fig. 3, Left). In contrast, the backbone (convex surface) of Zf-GRF is relatively unresponsive to ligand (Fig. 3, Right). Three ssDNA-binding clusters, denoted DNA-binding regions 1–3 (DBR 1–3), are identified with significant chemical shift perturbation on incubation with ssDNA (SI Appendix, Fig. S7A and B). Intriguingly, these regions include the conserved Zf-GRF basic residue clusters R473/K476/K477, which bind sulfate in our crystals, and R502 in the β2–β3 connecting loop, which we predict will interact with the DNA phosphate backbone. In addition, a clustering of conserved aromatic residues from β1 (V475), β2 (F486 and V488), and β3 (F506 and W508) display moderate ssDNA-induced chemical shift perturbation of the backbone amid. Together, these residues form a contiguous hydrophobic surface that may serve to base stack with the exposed ssDNA nucleobases (Fig. 3).

**Zf-GRF DNA Binding Is Critical for APE2 DNA Resection Functions.** To evaluate the functional significance of Zf-GRF DNA binding, we carried out mutational analyses of the Zf-GRF DNA-binding surface by specifically targeting the DNA interaction surface encompassing residues R473, K477, and R502. EMSA DNA-binding assays on ssDNA showed marked impairment of binding for all mutants tested (R473A, R473E, R502A, R502E, K477A, and K477E), consistent with a crucial role for this basic surface in mediating ssDNA interactions (SI Appendix, Fig. S8).

We next evaluated whether Zf-GRF DBR1–3 regions support APE2 nuclease function. To test this, we analyzed the PCNA-stimulated 3′-5′ nuclease activity of WT-APE2$^{25}$ on a 3′ recessed DNA substrate. Similar to human APE2 (31) and $S. cerevisiae$ Apn2 (41), in the absence of PCNA, *Xenopus laevis* APE2 (XIAPE2) carries out nuclease cleavage limited to one or two nucleotides from the 3′ end of a 5′ FAM-labeled substrate (Fig. 4A). The APE2 PIP box is known to promote PCNA binding (31, 36, 41). Accordingly, we observed robust stimulation of XIAPE2 nuclease activity by PCNA (Fig. 4, lane 3). We further found that, similar to human APE2 (27), XIAPE2 has a preference for the 3′ recessed ends over blunt-ended substrates, in both the presence and the absence of PCNA (SI Appendix, Fig. S9). Therefore, the Zf-GRF’s preference for binding ssDNA and ssDNA–dsDNA junctions correlates with full-length vertebrate APE2 substrate specificity.
Disruption of the Zf-GRF by mutations at R473, K477, or R502 impairs nucleolytic activity in the absence of PCNA, but under the conditions examined, partial PCNA-stimulated activity can be recovered for the R473E, K477A, K477E, and R502A mutants (Fig. 4, lanes 4–12). In contrast, the R502E mutation shows nearly complete loss of nucleolytic activity even in the presence of PCNA (Fig. 4, lanes 13 and 14). The R502E mutant does not significantly impair APE2 endonucleolytic incision of an apurinic site mimic (tetrahydrofuran) containing oligonucleotide (SI Appendix, Fig. S10). The purified R502E mutant protein is monomeric in solution as assessed by gel filtration, suggesting that this mutation does not result in gross unfolding and aggregation of APE2; however, an increase in the apparent molecular weight of this mutant as an MBP fusion (12% size increase), or for the purified Zf-GRF (10% increase) (SI Appendix, Fig. S11) suggests that in addition to mutating the DNA-binding surface, this variant may influence conformation of the Zf-GRF. Thus, overall our mutational work suggests that mutants that inhibit DNA binding in the Zf-GRF also impair PCNA-stimulated nucleolytic activity of APE2, underscoring a critical role for Zf-GRF DNA interactions in modulating APE2 DNA resection activity in vitro.

**APE2 Zf-GRF DNA Binding Is Important for the ATR-Chk1 DDR Pathway Activation in Oxidative Stress.** To determine the biological significance of APE2’s Zf-GRF in DNA binding and nucleolytic activity, we tested whether it is important for the oxidative stress-induced ATR-Chk1 checkpoint activation in *Xenopus* egg extracts. Consistent with our previous results (22), WT APE2 rescued H₂O₂-induced Chk1 phosphorylation in APE2-depleted Xenopus egg extracts (Fig. 4B). Furthermore, the initiation of DNA replication was also needed for activation of the ATR-Chk1 DDR pathway in oxidative stress (SI Appendix, Results and Discussion and Fig. S12A). Complementation of APE2-depleted egg extracts with a ΔZF APE2 protein that deletes the Zf-GRF failed to rescue the H₂O₂-induced Chk1 phosphorylation in APE2-depleted egg extracts, however (Fig. 4B). Further evaluation of chromatin-bound fractions showed that RPA32, ATR, ATRIP, and Rad9 were recruited to H₂O₂-damaged chromatin when WT APE2, but not ΔZF APE2, was added back to the APE2-depleted egg extracts. This occurred even though both WT APE2 and ΔZF APE2 were associated with H₂O₂-damaged chromatin (Fig. 4C). Histone H3 was used as a loading control for chromatin-bound fractions. These observations suggest that Zf-GRF-stimulated 3′-5′ exonuclease activity of APE2 is important for the generation of RPA-ssDNA; assembly of the checkpoint protein complex including ATR, ATRIP, and 9-1-1 complex; and subsequent Chk1 phosphorylation by activated ATR (22). Similarly, WT APE2, but not the Zf-GRF R502E nucleotide-deficient mutant, was able to rescue H₂O₂-induced Chk1 phosphorylation in APE2-depleted egg extracts (Fig. 4D). Although R502E APE2 was efficiently recruited to H₂O₂-damaged chromatin, WT APE2, but not R502E APE2, rescued the recruitment of RPA32, ATR, ATRIP, and Rad9 to H₂O₂-damaged chromatin in APE2-depleted egg extracts (Fig. 4D). Similar to the complete deletion of the Zf-GRF, these observations are consistent with a model in which the Zf-GRF...
DNA binding and regulation of nuclease resection play key roles in ATR-Chk1 checkpoint activation in oxidative stress.

We hypothesized that the high-affinity DNA interactions mediated by Zf-GRF, exogenous addition of purified Zf-GRF to egg extracts at a concentration similar to that of endogenous APE2 may modulate APE2 functions in a dominant-negative manner. The addition of purified Zf-GRF compromised H$_2$O$_2$-induced Chk1 phosphorylation (Fig. 4E), suggesting that the WT Zf-GRF motif may compete with endogenous APE2 for ssDNA in egg extracts. Notably, addition of the DNA-binding-deficient R502E mutant Zf-GRF to egg extracts had no effect on H$_2$O$_2$-induced peroxide-damaged chromatin (SI Appendix, Fig. S12B). These observations suggest that WT Zf-GRF may compete with endogenous APE2 binding to presumptive ssDNA regions on chromatin under oxidative stress conditions. Taken together, the foregoing results indicate that the WT Zf-GRF fragment functions in a dominant-negative manner to modulate the ATR-Chk1 pathway activation following oxidative stress.

**Discussion**

Our APE2 structure-function analyses reveal that APE2 contains an ordered Zf-GRF in its extreme C terminus, and that this region is critical for APE2 function in cellular responses to oxidative stress. Along with Zf-GRF, it has been established that the APE2-PCNA interface is mediated via a PIP-PCNA interaction (31, 36, 41). Structural order/disorder predictions, limited proteolysis, and SAXS results indicate that the intrinsically unstructured regions between the PCNA-binding PIP box and Zf-GRF flexibly connect the EEP–PIP–Zf-GRF architecture. The flexible connection of the PIP box to the catalytic domain and Zf-GRF appears suited to enable the Zf-GRF to tether ssDNA and direct the nuclease resection reaction (SI Appendix, Fig. S13).

Although the precise mode of molecular assembly of the APE2-PCNA-DNA complexes requires further investigation, we propose a model for the role and mechanism of the APE2 Zf-GRF in response to oxidative stress that involves the following: (i) APE2 is recruited to DNA damage with PCNA via its PIP box interaction and/or the Zf-GRF DNA sensing of ssDNA or DNA gaps (e.g., oxidative single-strand breaks); (ii) APE2’s Zf-GRF stabilizes in its 3′-5′ exonucleolytic resection activity through interaction with ssDNA or ssDNA/dsDNA junctions; and (iii) an extended stretch of exposed ssDNA is produced, facilitating RPA binding, checkpoint protein complex assembly, and ATR-Chk1 activation (SI Appendix, Fig. S13).

Disruption of either the APE2 Zf-GRF DNA-binding surface or its PCNA-binding PIP box (22) compromises downstream chromatin recruitment of RPA, checkpoint kinases, and checkpoint activation (SI Appendix, Fig. S13); however, deletion or mutation of the APE2 Zf-GRF has no detectable impact on APE2 association with chromatin following oxidative stress. We conclude that the catalytic domain and PIP box are necessary and sufficient for the recruitment of APE2 to DNA damage. Previous work has also demonstrated that the C terminus of budding yeast Apm2 binding both the PIP box and Zf-GRF is dispensable for a weak Apm2 AP-endonuclease activity, but is critically required for the repair of MMS-induced AP sites in vivo (42). Thus, we envisage that DNA binding and regulation of the nuclease reaction by the Zf-GRF, in combination with the PCNA–DNA interface is required to support robust APE2/Apm2 3′ end processing of DNA damage following oxidative stress and other forms of DNA damage.

Zn-finger domains participate in diverse functions, including peptide/protein binding, gene regulation, and lipid, RNA, and DNA binding (43–46). Zf-GRF-containing proteins are grouped as a distinct Pfam protein family (pfam.xfam.org/family/zf-GRF); however, DALI protein structural similarity searches show that the Zf-GRF is related to the eukaryotic RNA polymerase subunit RPB9, a member of the TFIIIS C-terminal Zn-ribbon superfamily (47–49) (pfam.xfam.org/family/PF01096) (SI Appendix, Fig. S14).

Although DALI failed to detect a structural relatedness of TFIIIS to APE2 Zf-GRF, manual overlays confirmed the structural relatedness of the overall topology of these domains (SI Appendix, Fig. S14 B and C). Interestingly, in the Zf-GRF family, the "GFP" consensus, along with a neighboring highly conserved "GPN" motif, structurally distinguishes APE2 from the TFIIIS family, and these sequence elements scaffold the DNA interaction surface loops and floor of the Zf-GRF DNA-binding groove and characteristic claw-like structure (SI Appendix, Fig. S14A). Thus, although clearly related to the TFIIIS Zn-ribbon, the structural divergence of the interstrand connector loops is tailored for nucleic acid binding functionality, possibly giving rise to distinct nucleic acid binding modes built on this common Zn-ribbon scaffold. An overall topological similarity to TFIIIS is also noted through the extended morphology of the N-terminal sequences flanking the APE2 Zf-GRF that adopt a polyproline extended-arm conformation in Zf-GRF, or extended structures in the TFIIIS-RNA polymerase II complex (SI Appendix, Fig. S14C), or the extended domain linker in the case of the RPB9 Zn-ribbon (SI Appendix, Fig. S14B). Notably, the "GRxF" motif, GPN, and β1-β2 connector loop basic DNA-binding motifs are well conserved in other DNA Zf-GRF damage response proteins, including the NEIL3 (Nei-like DNA glycosylase 3) DNA glycosylase and TOP3α (SI Appendix, Fig. S7B), indicating a possible conserved DNA-binding function in these human Zf-GRF-containing proteins.

This work establishes the Zf-GRF as a structure-specific DNA-binding element in regulating APE2 resection functions. This function highlights the diverse roles for Zn-finger DNA-binding domains in modulating the DDR. For example, recognition of DNA ends and nicks for reversal of adenylation DNA damage is dependent on structure-specific DNA binding the C$_2$H$_2$/C$_3$HE Aprataxin Zn-finger domain (50–52). For APE2, the Zf-GRF ssDNA-binding element is flexibly linked to the APE2 catalytic domain, yet is critical for robust catalytic activity. Conservation of the size and predicted disorder of the interdomain linkages in APE2 homologs from yeast to man suggests that this flexible association is needed for activity and/or dynamic regulation of APE2 activity. In line with this idea, an additional striking feature of the crystal lattice observed in our APE2 Zf-GRF structures is that the helical region (α1) of one protomer occupies the DNA-binding surface of the neighboring protomer (SI Appendix, Fig. S15). This helical contact surface occludes the hydrophobic cleft that we identify as important for binding ssDNA. We speculate that protein mimicry of Zf-GRF–ssDNA interactions might regulate APE2 Zf-GRF–DNA interactions and catalytic function, as has been observed in UNG–UGI interactions (53), among others (54). Taken together, our findings demonstrate that APE2 Zf-GRF is a distinct ssDNA-binding domain that facilitates the 3′-5′ resection of DNA damage for ATR-Chk1 DDR pathway activation following oxidative stress.

**Materials and Methods**

APE2 Protein Expression. The APE2$^{\text{WT}}$ and APE2$^{\text{R502E}}$ were PCR-amplified and subcloned into the pET MBP His6 LIC cloning vector (2Cc-T), containing a C-terminal MBP fusion tag (Addgene). The Zf-GRF domain was amplified and inserted into the pMCSG9 N-terminal MBP expression vector (MCSG). BL21-Al cells (Life Technologies) were transformed with all three protein constructs for protein expression overnight at 17°C in Terrific Broth. Induction of expression was carried out by the primary addition of 0.1% (v/vol), final concentration l-arabinose (GoldBio), followed by isopropyl β-D-thiogalactopyranoside.

APE2 protein purification, Zf-GRF crystallization data collection, structure solution and refinement, preparation of oligonucleotides substrates, electrophoretic mobility shift assays, fluorescence DNA-binding assays,
xenobase DNA damage assays, SAXS, and NMR methods are described in SI Appendix, Materials and Methods.

**X. laevis Egg Extracts and related Experiments.** The use and control of X. laevis were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Charlotte. X. laevis egg extracts were prepared as described previously (55, 56). The X. laevis egg extracts and related experiments with recombinant proteins and antibodies are described in SI Appendix, Materials and Methods.

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