Essential role for mammalian apurinic/apyrimidinic (AP) endonuclease Ape1/Ref-1 in telomere maintenance

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The major mammalian apurinic/apyrimidinic endonuclease Ape1 is a multifunctional protein operating in protection of cells from oxidative stress via its DNA repair, redox, and transcription regulatory activities. The importance of Ape1 has been marked by previous work demonstrating its requirement for viability in mammalian cells. However, beyond a requirement for Ape1-dependent DNA repair activity, deeper molecular mechanisms of the fundamental role of Ape1 in cell survival have not been defined. Here, we report that Ape1 is an essential factor stabilizing telomeric DNA, and its deficiency is associated with telomere dysfunction and segregation defects in immortalized cells maintaining telomeres by either the alternative lengthening of telomeres pathway (U2OS) or telomerase expression (BJ-hTERT), or in normal human fibroblasts (IMR90). Through the expression of Ape1 derivatives with site-specific changes, we found that the DNA repair and N-terminal acetylation domains are required for the Ape1 function at telomeres. Ape1 associates with telomere proteins in U2OS cells, and Ape1 depletion causes dissociation of TRF2 protein from telomeres. Consistent with this effect, we also observed that Ape1 depletion caused telomere shortening in both BJ-hTERT and in HeLa cells. Thus, our study describes a unique and unpredicted role for Ape1 in telomere protection, providing a direct link between base excision DNA repair activities and telomere metabolism.

genome stability | chromosome stability | endogenous DNA damage

Human cells must cope with a substantial number of spontaneous apurinic/apyrimidinic (AP) sites (1, 2). Noninstructional AP sites are mutagenic, and they can inhibit replication and transcription (3, 4). The human AP endonuclease Ape1, also called Ref-1, referring to a redox regulatory activity, is a crucial enzyme for the recognition and processing of AP sites in the base excision repair (BER) of DNA (5, 6). Ape1 has also been reported to possess a redox regulatory function (7, 8), and it is thought to function as a transacting factor in gene regulation (9–12). Recently, a novel role for Ape1 in RNA quality control has also been suggested (13, 14).

Mouse embryos deleted for the *APEX1* gene (encoding Ape1) die during embryogenesis (15–17). The accumulated evidence points to a vital role of Ape1 in the cell, but it has been challenging to explore the underlying molecular mechanisms. Attempts to dissect the essential domain(s) of Ape1 for cell survival have indicated the importance of both its DNA repair activity and two N-terminal lysine residues implicated in acetylation-mediated gene regulation, but not the Ref-1 redox activity (18, 19).

In this study, we report another function for Ape1: the protection of telomeres. Loss of Ape1 interferes with the association of telomeric repeat-binding factor (TRF)2 protein with telomeres, and the resulting telomere uncapping gives rise to genomic instability. The AP endonuclease and gene regulatory domains of Ape1 are essential for stabilization of telomeric DNA. Thus, this unpredicted role of Ape1 at telomeres provides a direct link between BER and telomere metabolism.

Results

Depletion of Ape1 Causes Multiple Mitotic Defects. Depletion of Ape1 protein in human cells or conditional deletion of the *APEX1* (*APE1*) gene in mouse cells blocks proliferation and promotes cell death (18, 19). To investigate the underlying molecular defects of the cell death linked to Ape1-deficiency, the morphology of Ape1-depleted U2OS cells was monitored at various times following release from a double-thymidine block. Whereas the proportion of apoptotic cells remained relatively small, the depletion of Ape1 by RNAi increased the percentage of cells with aberrant nuclei characterized by multilobular shape, micronuclei, and bi/multinuclei throughout the cell cycle (Fig. 1 *A* and *B* and Fig. S1 *A* and *B*). As a positive control for checkpoint defects, the depletion of Chk2 caused a gradual increase in apoptotic nuclei during the cell cycle (Fig. S1*B*). The accumulation of Ape1-deficient cells with multilobular nuclei, bi/multinuclei, and micronuclei is a hallmark of mitotic failure (Fig. 1 *A* and *B*). Although some percentage of apoptotic cells was evident both in U2OS and Saos-2 cells (Fig. S1*C*), the more prominent defect caused by Ape1 depletion seemed to be mitotic aberrations, suggesting that Ape1 plays a role in normal mitosis.

Rescue of Mitotic Defects in Ape1-Depleted Cells by Wild-Type and Domain-Specific Mutant Proteins. The multiple functions of Ape1 protein can be individually inactivated by site-specific mutations

Significance

Base excision repair (BER) is the predominant system correcting simple DNA base lesions formed by oxidation or other DNA-damaging agents. Repair of apurinic/apyrimidinic (AP) sites arising in the genome spontaneously or as intermediates of BER is critical owing to their toxic and mutagenic effects. Ape1/Ref-1 is the major AP endonuclease that initiates the processing of AP sites, allowing normal transcription and DNA synthesis to resume. In this study, we report a key role for Ape1/Ref-1 in telomere maintenance. Our findings suggest a direct link between BER and telomere dynamics, highlighting the potential contribution of oxidative DNA damage repair activities on telomere dysfunction in cancer, premature aging, or autoimmune diseases.

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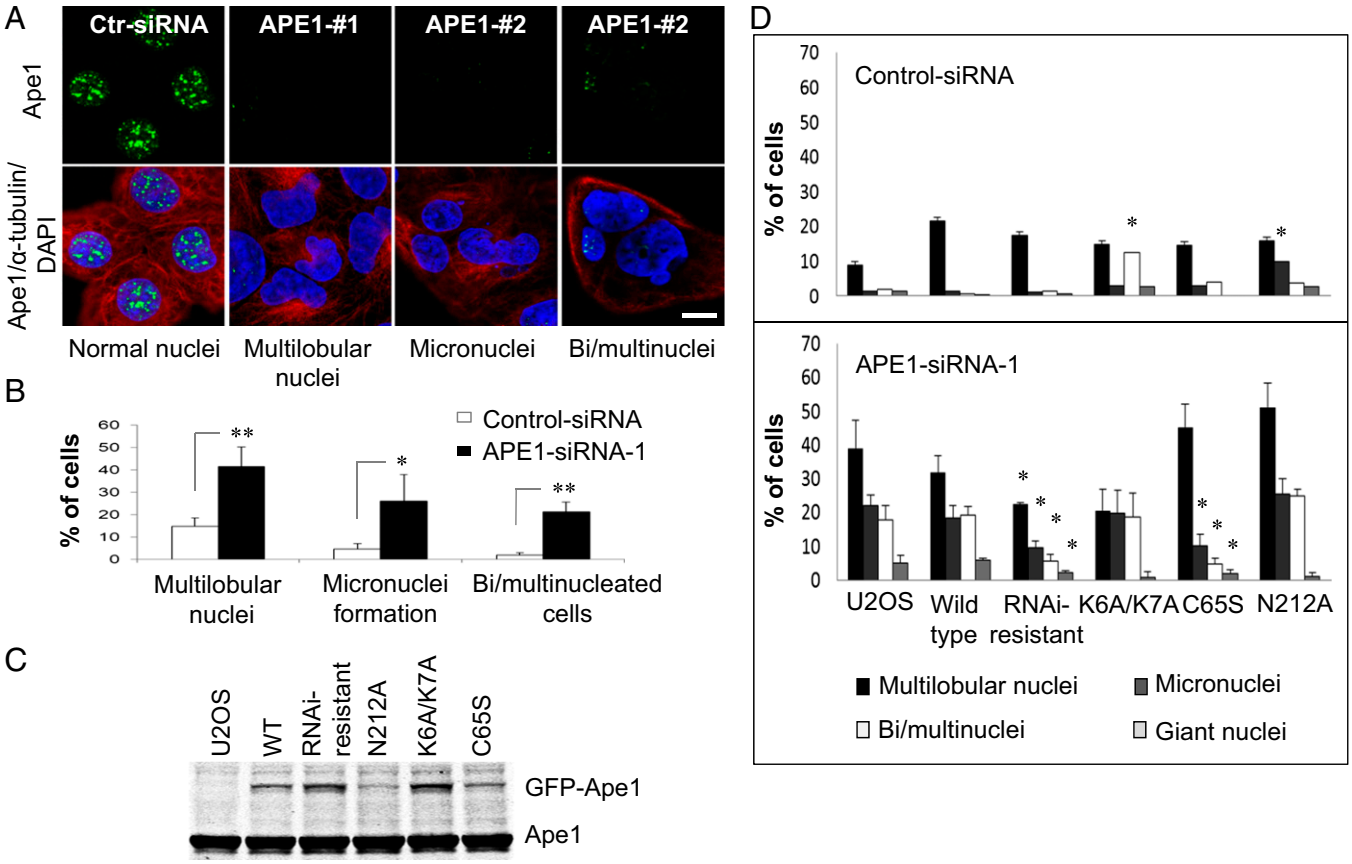

Fig. 1. Down-regulation of Ape1 is associated with mitotic defects in U2OS cells. (*A*) Depletion of Ape1 induces mitotic defects. U2OS cells treated with the indicated siRNAs were fixed at 72 h posttransfection and immunostained for Ape1 and α-tubulin. DNA was stained with DAPI. (Scale bar, 20 μm.) (*B*) Percentages of U2OS cells with multilobular nuclei, micronuclei, and bi/multinuclei were quantified at 72 h of the siRNA treatments. Error bars represent the SDs of three independent transfections. $*P \leq 0.05$; $**P \leq 0.01$ (Student *t* test). (*C*) Endogenous (Ape1) and GFP fusion variants of Ape1 (GFP-Ape1) were detected by immunoblotting in the virally transduced U2OS cells following five days of drug selection. (*D*) RNAi-resistant Ape1 and the C65S mutant Ape1, but not the K6A/K7A and the N212A mutant Ape1, partially rescued the mitotic defects of Ape1-depleted cells. U2OS cells expressing GFP-fusion variants of Ape1 were transfected with either control siRNA or APE1 siRNA-1 at day 5 of drug selection. Multilobular nuclei, micronuclei, bi/multinucleated cells, and giant cell nuclei were quantified at 72 h of the siRNA transfection. At least 100 cells were counted for each experiment, and the SDs of three independent experiments were calculated. Statistical significance between the cells expressing wild-type Ape1 and the cells expressing mutant forms of Ape1 is shown. $*P \leq 0.05$ (Student *t* test).

(Fig. S2*A*), allowing a genetic test of which activities are required for mitotic control. GFP fused to Ape1 was expressed in U2OS cells using lentiviral vectors directing RNAi-sensitive (wild-type) or RNAi-resistant transcripts (RNAi-resistant). In addition, the GFP-tagged RNAi-resistant forms of *APEX1* with mutations targeting the endonuclease (N212A) (20), gene regulation (K6A/K7A) (19, 21), or the Ref-1 redox functions (C65S) of Ape1 (22) were also expressed in U2OS cells (Fig. 1*C* and Fig. S2*B*). The C65S mutant form of Ape1 was detected in the cytoplasmic vesicles, with a relatively weak nuclear signal, indicating altered turnover of this protein (Fig. S2*B*). The pattern of subcellular localization of the N212A protein was similar to that for endogenous Ape1: mainly nuclear staining, with a focal accumulation in nucleoli (Fig. S2*B*). However, the K6A/K7A mutant showed a diffuse nuclear staining, with a minimal localization in nucleoli (Fig. S2*B*).

To address whether any mutant form(s) of Ape1 is able to rescue the Ape1 deficiency-associated mitotic defects, the cells expressing fusion Ape1 variants were transiently transfected with the control or APE1 siRNA-1 following a 5-d selection (Fig. 1*D* and Fig. S3). The percentage of cells with mitotic defects was quantified at 72 h of the siRNA treatment (Fig. 1*D*). In the control siRNA group, the expression of the K6A/K7A mutant caused a significant increase in the fraction of cells with bi/multinuclei, whereas the expression of the N212A provoked accumulation of the cells with micronuclei (Fig. 1*D*). In the APE1 siRNA-treated group, the RNAi-resistant expression of Ape1 partially rescued the cells depleted of endogenous Ape1 from mitotic defects, as evidenced by the decreased numbers of cells with aberrant nuclei (Fig. 1*D*). This partial rescue confirmed the biological activity of the fusion protein and was consistent with the lower expression of the GFP–Ape1 proteins relative to the endogenous Ape1 (Fig. 1*C*). In contrast, the expression of the K6A/K7A mutant, thought to be deficient in acetylation-dependent gene regulation by Ape1, also produced mitotic defects in Ape1-depleted cells, particularly promoting the formation of binucleated cells. Similarly, the expression of AP endonuclease mutant N212A, reported as deficient for AP endonuclease activity owing to a defect in DNA–AP site binding (20), generated increased numbers of cells with multilobular nuclei and micronuclei, as well as binucleated cells when endogenous Ape1 was suppressed (Fig. 1*D* and Fig. S3). However, the expression in Ape1-depleted cells of the C65S protein, proposed as defective for the redox function of Ape1, increased the numbers of cells with multilobular nuclei but did not increase the frequency of bi/multinucleated cells or micronuclei (Fig. 1*D* and Fig. S3). Thus, the endonuclease domain of Ape1 is required for successful mitotic progression, and there is also a role for lysines 6 and

7, perhaps through altered subcellular localization or post-transcriptional regulation.

Chromosome Missegregation and Telomere Dysfunction in Ape1-Depleted Cells. Errors in chromosome segregation can underlie defective mitosis. Following a cell-cycle block, Ape1 siRNA-treated U2OS cells showed a higher frequency of segregation defects, including misaligned chromosomes, lagging chromatin, and anaphase bridges (Fig. 2 *A* and *B*).

Unrepaired AP sites in Ape1-depleted cells are likely to stall DNA replication forks. Addressing the consequences of AP site accumulation in genome, we observed that depletion of Ape1 in U2OS, BJ-hTERT, and IMR90 primary fibroblasts caused chromosome fusions, chromosome fragmentation, rereplication, and broken chromosome arms (Fig. S4). These observations suggest that Ape1 deficiency promotes genomic instability at the chromosomal level, which results in segregation defects and mitotic failure.

Telomere-FISH assays in Ape1-depleted U2OS, BJ-hTERT, and IMR90 cells showed that Ape1 deficiency is associated with telomere dysfunction, including telomere signal loss, chromosome end fragmentation, chromosome end-to-end fusions, and extra telomeric signals (Fig. 2*C* and Fig. S4 *B–D*). These findings indicate that Ape1 is a critical factor for the stabilization of telomeric chromatin. It is worth noting that these three human cell lines represent three different effects on telomeres: U2OS cells use the recombination-dependent alternative lengthening of telomeres (ALT) pathway (23), BJ-hTERT cells express telomerase (24), and the telomeres of IMR90 cells gradually erode with increasing cell divisions (25).

To address the role of Ape1 in telomere maintenance in another species, Ape1 was down-regulated in NIH 3T3 mouse embryonic fibroblasts. Partial depletion of Ape1 (~50%) with synthetic mouse-specific APE1 siRNAs caused chromosome abnormalities, including formation of radial chromosomes and end-to-end chromosome fusions (11%, $n = 72$) (Fig. S5). These results suggest that Ape1 functions in a conserved biological pathway that is critical for protection of both human and mouse telomeres.

DNA Repair and Gene Regulatory Domains of Ape1 Are Essential for Its Role at Telomeres. To map the domains of Ape1 essential for its telomere function, wild-type or mutated forms of Ape1 were expressed in U2OS and BJ-hTERT cells. FISH analyses of the chromosomes using telomere G-strand or C-strand peptide nucleic acid (PNA) probes showed that expression of the K6A/K7A or N212A protein, but not of the C65S protein, increased the frequency of end-to-end fusions in both U2OS (Fig. 2*D*) and BJ-hTERT cells (Fig. S6). These results suggest that both the endonuclease domain of Ape1 and the acetylatable N-terminal lysines are required for telomere stabilization, and the effects are observed in both ALT-dependent and telomerase-expressing cells.

Reduced Presence of TRF2 at Telomeres of Ape1-Depleted Cells. Telomeres are tracts of G-rich nucleotide sequences, and the formation of oxidized bases and AP sites may thus be more frequent at telomeres than elsewhere in the DNA (26–28). To test whether AP site accumulation at telomeres alters the dynamics of telomere-associated proteins, we investigated the localization of TRF2 in APE1 siRNA-treated cells. This

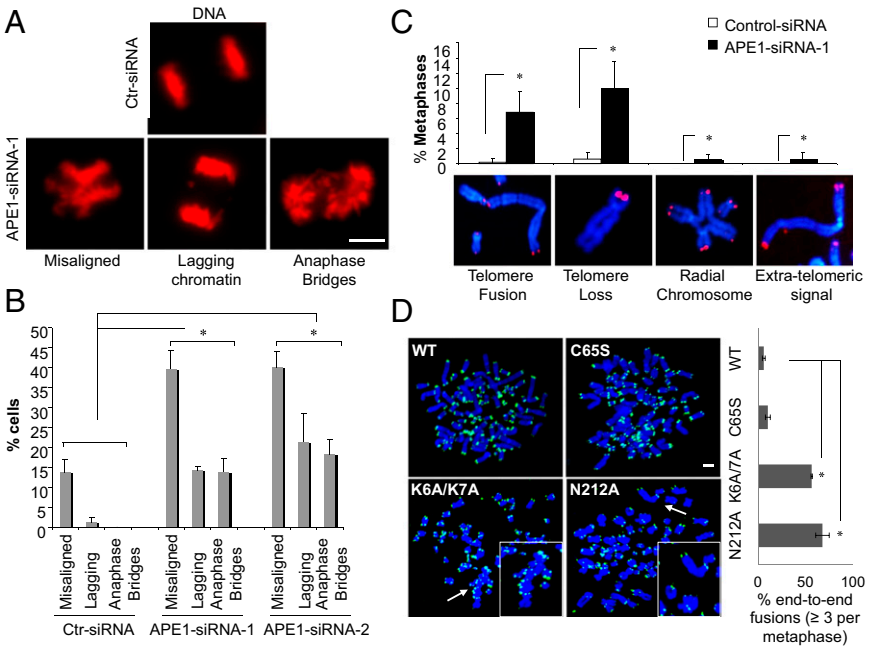

Fig. 2. Ape1 depletion causes chromosome segregation defects and telomere dysfunction. (*A*) Chromosome segregation defects in Ape1-depleted cells. U2OS cells were transfected with control (Ctr.) or APE1 siRNAs and synchronized by double thymidine treatment. At day 3, the cells were released into fresh medium and fixed after 9 h. DNA was stained with PI. (Scale bar, 10 μm.) (*B*) The percentage of cells representing three examples of typical chromosome segregation defects is shown. Error bars denote the SDs of three independent experiments. *$P \leq 0.05$ (Student *t* test). (*C*) Telomere aberrations in Ape1-depleted cells. U2OS cells treated with control or APE1 siRNAs were synchronized by the double thymidine treatment, and released into nocodazole (100 ng/mL) for 16 h (Fig. S4). Telomeres were labeled with a PNA probe (red). Representative images of telomere aberrations observed in Ape1-depleted cells are shown. At least 50 metaphases per experiment were counted, and SDs were calculated from three independent transfections. *$P \leq 0.01$ (Student *t* test). (*D*) The DNA repair and gene regulatory domains of Ape1 are required for telomere protection. U2OS cells were infected with the viruses expressing the wild-type (WT) or the mutant forms of Ape1: K6A/K7A, C65S, and N212A. Following a 7 d-drug selection, metaphase chromosomes were prepared, and telomeres were labeled with a PNA telomere C-probe (green). DNA was stained with DAPI. (Scale bar, 5 μm.) From three independent infections, at least 50 metaphases per experiment were analyzed, and metaphases showing three or more end-to-end chromosome fusions were quantified. *$P \leq 0.01$ (Student *t* test).

analysis showed that depletion of Ape1 reduced the amount of TRF2 at telomeres in both U2OS (Fig. 3*A*) and BJ-hTERT cells (Fig. S7*A*). A telomere-ChIP assay in U2OS cells showed that telomeric sequences were enriched following chromatin immunoprecipitation by an Ape1 antibody, and that enrichment was reduced upon treatment of cells with APE1 siRNAs (Fig. 3*B*). Ape1 depletion also reduced the recovery of telomeric DNA with a TRF2 antibody (Fig. 3*B*) without diminishing TRF2 protein in these cells (Fig. S7*B*). Evidently, the loss of Ape1 disrupts the association of TRF2 with telomeres, again regardless of the telomere maintenance pathway.

Loss of Ape1 Induces DNA Damage Response at Telomeres. Loss of telomere protection by TRF2 leads to end-to-end chromosome fusions and induces DNA damage responses (29). To test whether Ape1 deficiency-associated dislocation of TRF2 from telomeres initiates DNA damage responses, BJ-hTERT fibroblasts were treated with APE1 siRNAs, and dual-stained for TRF1 and the phosphorylated histone γH2AX (Fig. 3*C*). Although Ape1 suppression did not significantly stimulate γH2AX foci formation in BJ-hTERT cells, the colocalization of γH2AX with TRF1 in distinct foci was induced (Fig. 3*C*), indicative of the activation of DNA damage responses at telomeres.

Ape1 Associates with the Telomere Protein Complex. Asynchronously growing cells did not show significant numbers of coincident Ape1 and TRF2 foci, but colocalization was observed specifically in the S-phase of the synchronized U2OS cells (Fig. 4 *A* and *B*). The percentage of cells showing more than three colocalizing Ape1/TRF2 foci remained around 6% (±1.0) in the early S-phase at 3 h after the release from thymidine but reached 21.4% at 6 h (± 2.6) and 10.7% at 9 h (± 1.6) (Fig. 4*B*). These results indicate that Ape1 functions in DNA repair of telomeres during the synthesis of DNA.

Supporting these data, TRF2 coimmunoprecipitated with Ape1 in HEK293 and U2OS cells (Fig. 4*C* and Fig. S8*A*), and this association was diminished by methyl methane sulfonate (MMS) treatment, perhaps due to the recruitment of Ape1 to MMS-induced damage sites throughout the genome. In addition, Ape1 coincided with POT1, the single-stranded telomere-binding protein, in a fraction of asynchronously growing BJ-hTERT and U2OS cells, and Ape1/POT1 colocalization was enhanced in response to MMS (Fig. 4*D* and Fig. S8*B*).

Finally, we measured telomere length in Ape1-depleted U2OS, BJ-hTERT, and HeLa cells. Suppression of Ape1 in BJ-hTERT cells resulted in shortening of the terminal telomere fragments from a mean length of 15.7 kb to 14.0 kb during just 3 d of siRNA treatment (Fig. S8*C*). Similarly, the terminal telomere fragments of HeLa cells were also shortened from a mean length of 5.9 kb to 5.3 kb (Fig. S8*D*). This short-term assay did not reveal a clear size change in the telomeres of Ape1-depleted U2OS cells, but their greater initial length precluded an accurate measurement of the mean length.

Discussion

The removal of DNA lesions is particularly important for DNA synthesis, and the replication of a damaged DNA template can have mutagenic or lethal consequences (30). We previously reported that prolonged Ape1 depletion results in the accumulation of AP sites in genomic DNA associated with increased levels of apoptosis (18). Under the experimental conditions of the current study using an RNAi system, with residual Ape1 activity, the ratio of apoptotic cell death in Ape1-depleted U2OS cells (p53-intact) and Saos-2 cells (p53-null) was 15% and 12.9%, respectively (Fig. S1*C*). Surprisingly, the majority of these Ape1-depleted, ALT-dependent cells could progress into mitosis (Fig. S1*B*). However, mitosis in the Ape1-depleted cells was defective.

Our detailed analysis of the aberrant mitoses associated with Ape1 deficiency showed that the telomere defects observed in

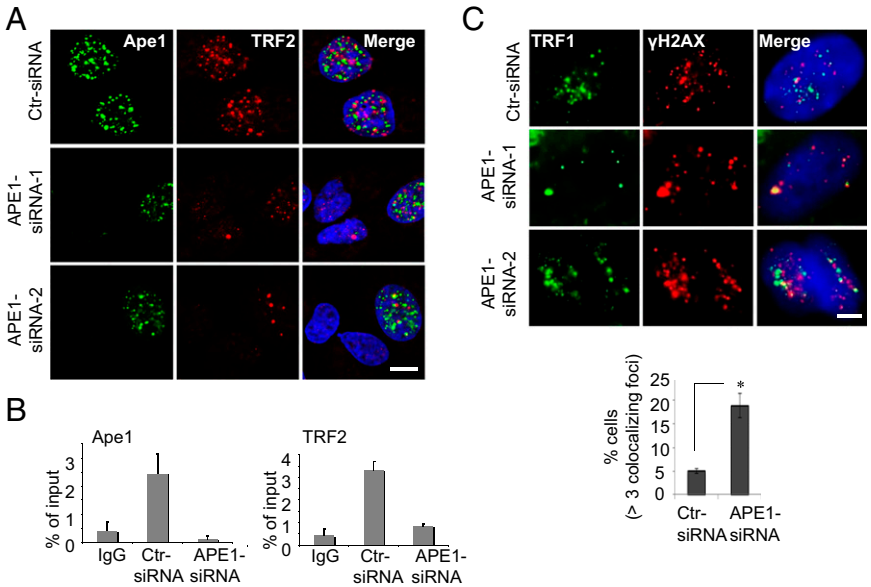

Fig. 3. Ape1 is required for stabilization of telomeric DNA. (*A*) Ape1 depletion causes TRF2 loss at telomeres. Control (Ctr.) and APE1 siRNA transfected U2OS cells were fixed at 72 h posttransfection and immunostained for Ape1 (green) and TRF2 (red). DNA was stained with DAPI. (Scale bar, 10 μm.) (*B*) Telomere-ChIP assay was performed in U2OS cells at 72 h of siRNA transfection. The recovered DNA from chromatin immunoprecipitation with Ape1 or TRF2 antibodies was submitted to quantitative real-time PCR analysis. Telomeric DNA was amplified using a telomere-specific primer pair, and the results are presented as the percentage of input DNA. Error bars correspond to SDs from three independent experiments. (C) Ape1 depletion induces DNA damage response at telomeres. BJ-hTERT cells transfected with control (Ctr.) or APE1 siRNAs were fixed at 72 h posttransfection and immunostained for TRF1 (green) or γH2AX (red). DNA was stained with DAPI. (Scale bar, 5 μm.) Cells showing more than three TRF2/γH2AX colocalizing foci from three independent, blinded experiments were quantified by counting at least 100 cells per experiment. *$P \leq 0.01$ (Student *t* test).

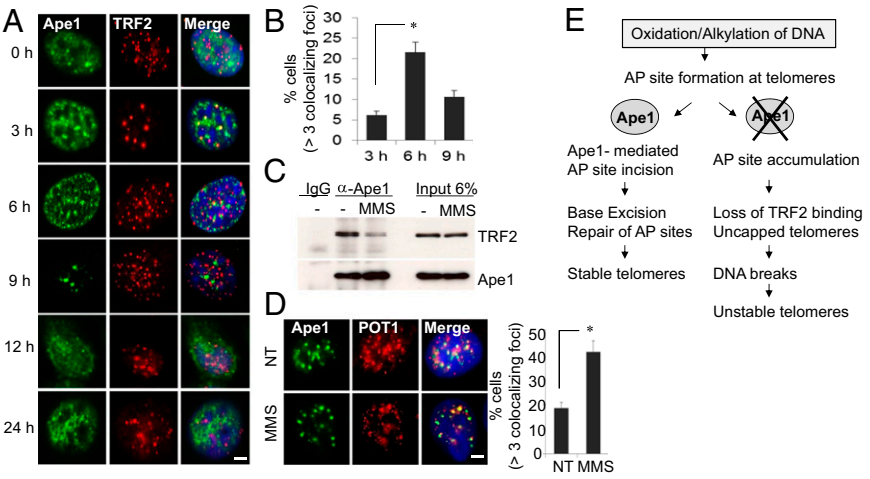

Fig. 4. Ape1 associates with TRF2 and POT1 in the cell. (*A*) Cell cycle-specific association of Ape1 with TRF2. U2OS cells were synchronized by double thymidine treatment and released into fresh medium. Cells were fixed at the indicated time points and immunostained for Ape1 (green) and TRF2 (red). DNA was stained with DAPI. (Scale bar, 5 μm.) (*B*) Quantification of cell cycle-specific association of Ape1 and TRF2. At least 100 cells per experiment were counted, and cells showing more than three Ape1/TRF2 colocalizing foci were quantified from three independent experiments. *$P \leq 0.01$ (Student *t* test). (*C*) Ape1 forms a complex with TRF2 in the cell. Nontreated or MMS-treated (15 μg/mL, 16 h) HEK293 cell extracts were subjected to immunoprecipiation with an anti-Ape1 antibody. Immunoprecipitates were then immunoblotted for TRF2 and Ape1. (*D*) Ape1 colocalizes with POT1 in unperturbed BJ-hTERT cells. Cells were either left nontreated (NT) or treated with MMS (15 μg/mL, 16 h). The cells were then fixed and immunostained for Ape1 (green) and POT1 (red). DNA was stained with DAPI. (Scale bar, 5 μm.) At least 100 cells per experiment were counted, and cells with more than three Ape1/POT1 colocalizing foci were quantified from three independent experiments. *$P \leq 0.01$ (Student *t* test). (*E*) Mechanism of Ape1 deficiency-associated telomere dysfunction. Our genome is constantly exposed to oxidation as a consequence of routine cell metabolism. Extrinsic factors add further to this load. Upon removal of modified bases from DNA, AP sites form at telomeres and are rapidly processed by Ape1 to direct BER of telomeric lesions. Under Ape1 deficiency, AP sites accumulate at telomeres, causing TRF2 dissociation and telomere uncapping. Unprocessed AP sites are exposed to nuclease attack or converted to DNA breaks during replication. Upon initiation of DNA damage responses, improper repair of damaged telomeres results in telomere fusion and degradation.

Ape1-depleted cells are the likely cause of the elevated levels of chromosome missegregation and aneuploidy. Apparently, as little as 50% of the normal Ape1 complement is sufficient for this role, because no aberrant mitosis has been reported in mice heterozygous for *APEX1*, which contain only half the normal protein level (17). Mouse cells carrying severe telomere defects are likely to be eliminated owing to a strong cell-cycle checkpoint response. Thus, a good deal of the inviability of *APEX1*-null cells may arise through mitosis, owing to the severe telomere defects preventing proper chromosome segregation. Because mouse cells have both longer telomeres and higher telomerase activity than human cells, telomere aberrations caused by partial depletion of Ape1 may require more cell divisions before mouse telomeres reach a critically short telomere length (31). Although we did not duplicate all our studies in mouse models, depletion of Ape1 in NIH 3T3 mouse cells caused the same range of chromosome defects (Fig. S5) seen in human cells. Thus, Ape1 is critical for the stability of mouse telomeres. It is worth noting that the deletion of *Saccharomyces cerevisiae OGG1,* encoding a DNA glycosylase for oxidized guanines, was reported to cause the accumulation of 8-oxoguanine in telomeric DNA and altered telomere length (32), which points to a role for BER in telomere maintenance in yeast.

A possible mechanism for the role of Ape1 at telomeres can be envisioned as follows (Fig. 4*E*): Accumulation of lesions in telomeric DNA interferes with the recruitment of factors required for telomere protection, consistent with the loss of TRF2 protein from telomeres in Ape1-depleted cells. We note that an in vitro study showed that the binding of TRF1 and TRF2 to synthetic telomeric DNA was disrupted by the presence of AP sites (33). The role of Ape1 at telomeres could be more than just a DNA repair function. Ape1 loss is likely to cause a distortion in the telomeric load of telomere binding proteins, which further exposes telomeres to error-prone repair processes, resulting in end-to-end fusions and telomere degradation.

Ape1 was detected in a general screen for telomere-associated proteins in HeLa cells (34). Our telomere-ChIP assays in U2OS cells did confirm the presence of Ape1 at telomeres under normal growth conditions. Hence, the presence of Ape1 at telomeres may reflect the amount of BER running in telomeres during routine cell metabolism. Supporting our finding that Ape1 associates with the members of the telomere complex such as TRF2 and POT1, Ape1 was identified as a protein interacting with the core telomere proteins including TRF2 (35). Furthermore, shelterin proteins TRF1, TRF2, and POT1 have been recently shown to stimulate Ape1 endonuclease activity on a telomeric abasic substrate in vitro (36).

Our studies of the expression of specific mutant forms of Ape1 support a critical role of the AP endonuclease activity in telomere protection. Despite relatively low levels of mutant protein expression, aberrant chromosomes (end-to-end fusions and chromosome fragments) were evident in metaphase spreads of cells expressing the AP endonuclease-deficient Ape1 protein (Fig. 2*D* and Fig. S6). Intriguingly, cells expressing the K6A/K7A mutant also showed severe chromosome fusions (Fig. 2*D* and Fig. S6). These results were unexpected, because these two lysine residues are remote from the AP endonuclease domain and do not affect its activity in vitro (19). However, the K6A/K7A protein does have defective subcellular localization, so these residues may affect the recruitment of Ape1 to important nuclear sites such as telomeres. The first 35 N-terminal residues of Ape1 are not represented in the available crystal structure of the protein (37), and we cannot exclude the possibility that altered gene regulation dependent on acetylation of lysines 6 and 7 might indirectly affect telomere protection and genome stability. Unlike the K6A/K7A protein, the redox-defective C65S Ape1 protein did not stimulate the formation of chromosome fusions (Fig. 2*D* and Fig. S6), suggesting that the C65S mutant does not interfere with the telomere function of Ape1.

There is a consensus from various studies indicating oxidative stress as a cause of telomere shortening (38–40). It is less clear

how well oxidative damage is repaired in telomeric DNA, or whether this repair might be compromised by or contribute to telomere shortening under chronic oxidative stress. Although BER is expected to be the predominant pathway for repairing oxidative DNA damage, no direct link has been reported between mammalian BER deficiency and telomere dysfunction. Such investigations are complicated by the cell- or organism-essential functions of the core BER proteins Ape1, Polβ, FEN1, and XRCC1. This study shows that partial depletion of Ape1 resulted in telomere shortening in two different human cell lines, BJ-hTERT and HeLa (Fig. S8 *C* and *D*), both expressing telomerase to maintain telomeres. Although further research is needed to understand the exact mechanism of Ape1 deficiency-associated telomere shortening, it is likely that shortening of telomeres in Ape1-depleted cells is a consequence of dislocation of protective protein complexes from telomeres and subsequent faulty repair of damaged DNA ends. Our findings provide at least one mechanistic approach to understand how oxidative DNA damage affects genomic stability and cell survival through telomere dynamics and the relation of this to telomere dysfunction in cancer, premature aging, or autoimmune diseases.

Experimental Procedures

Cell culture and chemicals, transient knockdown of Ape1 protein expression, generation of RNAi-resistant Ape1 and its domain-specific mutations, Western blot and coimmunoprecipitation studies, immunofluorescence, telomere FISH assay, telomere ChIP assay, and telomere length analysis are discussed in *SI Experimental Procedures*.

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