Regulation of WRN Helicase Activity in Human Base Excision Repair*

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Werner syndrome patients are deficient in the Werner protein (WRN), which is a multifunctional nuclear protein possessing 3′→5′ exonuclease and ATP-dependent helicase activities. Studies of Werner syndrome cells and biochemical studies of WRN suggest that WRN plays a role in several DNA metabolic pathways. WRN interacts with DNA polymerase β (pol β) and stimulates pol β strand displacement synthesis on a base excision repair (BER) intermediate in a helicase-dependent manner. In this report, we examined the effect of the major human apurinic/apyrimidinic endonuclease (APE1) and of pol β on WRN helicase activity. The results show that WRN alone is able to unwind several single strand break BER intermediates. However, APE1 inhibits WRN helicase activity on these intermediates. This inhibition is likely due to the binding of APE1 to nicked apurinic/apyrimidinic sites, suggesting that APE1 prevents the promiscuous unwinding of BER intermediates. This inhibitory effect was relieved by the presence of pol β. A model involving the pol β-mediated hand-off of WRN protein is proposed based on these results.

Werner syndrome (WS) is an autosomal recessive disorder manifested by premature aging and the early onset of age-related diseases (1). WS patients carrying null alleles of WRN display a remarkable number of clinical signs and symptoms associated with normal aging at an earlier stage of life, including graying and loss of hair, cataracts, dermal atrophy, diabetes mellitus (type II), osteoporosis, atherosclerosis, and cancers (1, 2).

Cells from WS patients are characterized by elevated genome instability and hypersensitivity to DNA-damaging agents. WS cells have frequent chromosomal deletions, translocations, and other complex rearrangements (3, 4). Hypersensitivity to DNA-damaging agents that generate reactive oxygen species such as 4-nitroquinoline-1-oxide (5, 6) and ionizing radiation (7, 8) has been observed, suggesting that WRN may participate in some aspect of oxidative DNA damage repair such as base excision repair (BER) (5, 6, 9). Since phenotypic changes with aging have been hypothesized to be attributed to the accumulation of oxidative products, the involvement of WRN in BER may contribute to the premature aging seen in WS patients.

BER contains two subpathways, short patch and long patch BER (10–16). The first steps of BER are common to both subpathways. A DNA lesion is recognized by a damage-specific DNA N-glycosylase, which releases the damaged base moiety, and the DNA backbone is then typically cleaved by AP endonuclease 1 (APE1). In short patch BER, DNA polymerase β (pol β) subsequently adds a single nucleotide at the resulting gap followed by terminal processing and the broken DNA strand is ligated to complete repair. In long patch BER, pol β carries out strand displacement synthesis starting from the APE1-induced strand break, displacing 2–10 nucleotides before the 5′-protruding flap is cleaved by flap endonuclease 1 (FEN-1) and the DNA strand is resealed by DNA ligase. This pathway involves other replicative proteins as well such as proliferating cell nuclear antigen, replication protein A (RPA), and DNA polymerase δ (pol δ). Some evidence suggests that BER is a coordinated process in which BER intermediates are passed from one enzyme to the next in the pathway (17, 18, 19), such that the cell is protected from the potentially cytotoxic and mutagenic effects of BER DNA intermediates. Recently, a NEILs (Nei-like/poly nucleotide kinase-dependent BER pathway) was reported (20). NEILs acts as the initial glycosylase on certain base damages, generating a 3′-phosphate terminus, and then polynucleotide kinase removes this 3′-blocking group prior to repair synthesis and ligation.

Extracts from WS cells efficiently repaired a plasmid containing a hydrolytic (natural) abasic (AP) site in vitro and are thought to be proficient in short patch BER (21). In contrast, biochemical studies demonstrate that WRN interacts physically and/or functionally with long patch BER proteins including pol δ, proliferating cell nuclear antigen, RPA, and FEN-1 (3). WRN interacts physically with FEN-1 and stimulates its DNA flap cleavage activity (22, 23). As deficiencies in the removal of the 5′-deoxyribose phosphate (dRP)-containing flap could potentially lead to genome instability (24), the stimulation of FEN-1 activity by WRN suggests that WRN may participate in long patch BER. In addition, we recently demonstrated that wild-type WRN stimulates pol β strand displacement DNA synthesis on a nicked BER intermediate in vitro (25). This reaction requires an active WRN helicase domain and involves unwinding of the nicked BER intermediate by WRN, suggesting that displacement of the downstream
strand may be important for long patch BER. These findings are consistent with the possible role of WRN in long patch BER.

This study examines WRN helicase activity on BER intermediates in the absence or presence of BER proteins. The results show that WRN unwinds BER intermediates in the absence of other BER factors. We also demonstrate that WRN physically interacts with APE1 and that APE1 inhibits WRN unwinding of BER intermediates. This inhibition is alleviated by the presence of pol β. The implication of these results for the regulation of BER is discussed.

**EXPERIMENTAL PROCEDURES**

**Materials—**Terminal deoxynucleotidyltransferase was purchased from New England Biolabs. [α-32P]dATP was obtained from Amersham Biosciences. Oligonucleotides were obtained from Midland Certified Reagent Company (Midland, TX).

**Proteins—**Wild-type WRN (25, pol β (26), and uracil DNA glycosylase (UDG) (27) were purified as described previously. The H390N APE1 (a generous gift of Dr. Bruce Demple, Harvard School of Public Health), APE1Δ35, and the full-length APE1 were purified essentially as described (28). The heterotrimer of human RPA was generously provided by Dr. Mark Kenny (Albert Einstein Cancer Center, Bronx, NY). Recombinant GST-WRN fusion proteins were overexpressed in *Escherichia coli* and purified using glutathione beads (Amersham Biosciences) as described previously (29).

**DNA Substrates—**The appropriate oligonucleotide (10 pmol, Fig. 1A) was 3'-radiolabeled with [α-32P]dATP and terminal deoxynucleotidyltransferase according to the manufacturer’s instructions. Unincorporated nucleotides were removed using a G-25 Sephadex spin column (Amersham Biosciences). The radiolabeled oligonucleotide was then annealed to the appropriate template oligonucleotide (25 pmol) by heating in 50 mM NaCl at 95 °C for 5 min followed by cooling down to room temperature (22 °C). If necessary, an upstream oligonucleotide (35 pmol) was then annealed to the duplex substrate by heating for 1 h at 37 °C and slowly cooling to 22 °C.

To prepare AP substrates, the uracil-containing DNA substrate (34 units, 1250 fmol) was pretreated with UDG (a final concentration of 2.5 U/ml) at 37 °C for 5 min followed by the addition of 30 U/ml TRF2 and 30 U/ml RPA (35, 36). This analysis confirms that the APE1-treated DNA has a 5'-dRP terminus and that 5'-dRP is the only detectable 5'-phosphate group (35, 36). This analysis confirms that the APE1-treated DNA has a 5'-dRP terminus and that 5'-dRP is removed by the 5'-phosphate lyase activity of pol β. An F-nick substrate is a nicked DNA containing a tetrahydrofuran (termed F) at the 5'-end of the labeled 19-mer oligonucleotide (Fig. 1B, lane 5).

To address whether WRN alone could unwind BER intermediates following the removal of all of the BER proteins by indirect immunofluorescence and analyzed as described in Partridge et al. (34). Rabbit anti-APE1 was purchased from Trevigen, monoclonal anti-WRN antibody was obtained from BD Transduction Laboratories (San Diego, CA), and anti-TRF2 was from Imgenex (San Diego, CA). Cy3-conjugated secondary monoclonal antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Alexa 488-conjugated secondary antibodies and the DNA stain 4',6-diamidino-2-phenylindole dihydrochloride were purchased from Molecular Probes (Eugene, OR).

**Helicase Assay—**Proteins and radiolabeled DNA substrates were incubated in helicase reaction buffer (50 mM HEPES, pH 7.5, 20 mM KCl, 4 mM MgCl2, 2 mM ATP, 2 mM dithiothreitol, 0.1 mg/ml BSA) in a final volume of 10 μl. Reactions were incubated at 37 °C for 15 min and terminated by the addition of 3X stop dye (0.05 mM EDTA, 40% glycerol, 1% SDS, 0.05% bromphenol blue, 0.05% xylene cyanol) to a final concentration of 1X stop dye. Products were run on a 12% native polyacrylamide gel, visualized using a PhosphorImager, and quantitated using ImageQuant software.

**Fluorophoretic Mobility Shift Assay—**Protein was incubated with 5'-32P-labeled duplex DNA substrates (10 fmol) for 15 min at 0 °C in 10 μl of BER buffer, and the binding reactions were subsequently analyzed on an 8% non-denaturingpolyacrylamide gel in running buffer (20 mM Tris-HCl, pH 7.2, 10 mM NaOAC, 0.5 mM EDTA) at 4 °C. Bands were visualized using a PhosphorImager and quantitated using ImageQuant software.

**RESULTS**

**WRN Alone Unwinds BER Intermediates—**We recently found that WRN unwinds the downstream strand at a nicked AP site in the presence of BER proteins (25). This finding suggests that WRN may interact specifically with BER intermediates and/or BER protein complexes and that these interactions may regulate WRN helicase activity. This idea was investigated by carrying out WRN helicase assays with BER intermediates in the absence or presence of BER proteins.

A 3'-end-labeled 34-mer duplex oligonucleotide containing a uracil at position 18 was treated with human UDG to generate a DNA substrate with an AP site (see Fig. 1A for substrates). The AP site-containing DNA was incubated with human APE1 to generate an incised AP site substrate (dRP-nick) with a 5'-dRP and a 3'-hydroxyl terminus. To verify the nature of the DNA substrates, they were reduced with NaBH4 or treated with human pol β and analyzed on a 20% sequencing polyacrylamide gel (Fig. 1B). The NaBH4-treated DNA is a 5'-dRP-18-mer, which migrates slightly faster than a 5'-phosphate 19-mer (p19), but slower than p18. The pol β-treated DNA (Fig. 1B, lane 2) migrates with p18 as this enzyme excises the 5'-dRP group (35, 36). This analysis confirms that the APE1-treated DNA has a 5'-dRP terminus and that 5'-dRP is removed by the 5'-phosphate lyase activity of pol β. An F-nick substrate is a nicked DNA containing a tetrahydrofuran (termed F) at the 5'-end of the labeled 19-mer oligonucleotide (Fig. 1B, lane 5).

To address whether WRN alone could unwind BER intermediates following the removal of all of the BER proteins by phenol/chloroform extraction, the above substrates were incubated with various concentrations of WRN under helicase reaction conditions. As shown in Fig. 2, A and B, WRN efficiently unwound duplex DNA containing a 1-nucleotide gap, an F-nick, a dRP-nick, or a 3'-overhang. At 4 nM WRN, ~70% labeled downstream primer of the four substrates was displaced. In contrast, blunt-ended duplex DNA containing an intact AP site was not a substrate for the WRN helicase (Fig. 2B). These findings demonstrate that BER strand break intermediates are substrates for WRN and suggested a potential role for WRN helicase activity in BER. We note that WRN unwinds nicked DNA substrates for WRN and suggested a potential role for WRN helicase activity in BER.
DNA with a 5’-dRP terminus or with a 5’-phosphate terminus produced following pol β dRP lyase activity on a 5’-dRP-nicked DNA substrate (data not shown). WRN helicase also unwinds dRP-nicked DNA in the presence of a dRP lyase-defective pol β (data not shown).

WRN Unwinding of BER Intermediates Is Inhibited by APE1—There are ~300,000 molecules of APE1/mammalian cell. Thus, APE1 is present in large excess over DNA glycosylases and pol β (37, 38). The high level of APE1 may be required to efficiently repair the estimated 10,000 AP sites that accumulate in the mammalian genome per day (39).

The effect of APE1 on WRN helicase activity was examined by carrying out WRN helicase assays with dRP-nick and F-nick DNA substrates in the presence of APE1. The results show that WRN-catalyzed unwinding of all of the BER substrates decreased in the presence of increasing amounts of APE1 (Fig. 3, A and B), whereas WRN was able to effectively unwind the 19-mer downstream primers of the dRP-nick and F-nick substrates in the absence of APE1. The inhibition was severe on the F-nick substrate compared with the dRP-nicked substrate, even at lower concentrations of APE1 (4 nM) (Fig. 3B). In contrast, APE1 (up to 8 nM) did not affect WRN helicase activity on forked or 5’-recessed duplex DNA (Fig. 3C). APE1 is not expected to bind to forked or 5’-recessed duplex DNA with high affinity (40, 41), suggesting that specific interactions between APE1 and the DNA substrates are required for APE1 to inhibit WRN helicase activity. It is likely that a stable protein/DNA complex between APE1 and a BER intermediate protects DNA termini from undesired processing by other proteins. This finding suggests that the mechanism by which APE1 inhibits WRN is based on steric hindrance at AP-nick termini.

WRN Interacts with APE1—APE1 inhibition of WRN helicase activity may arise from preventing access to the strand break site and/or inactivating the enzyme through a direct physical association. Therefore, we explored whether APE1 and WRN directly interact. Initial studies using a pull-down assay with a C-terminal WRN fragment (GST-WRN949–1432) and a HeLa NE found that WRN co-precipitates with APE1 from HeLa NE (Fig. 4A, lane 3). The association of APE1 is specific with GST-WRN949–1432, as GST alone did not precipitate APE1 (Fig. 4A, lane 2). This finding suggests that WRN and APE1 may interact physically in vivo.

To assess whether these two proteins directly interact, direct and indirect ELISAs were performed with purified WRN and APE1 (Fig. 4B). When microtiter wells were coated with APE1 (Fig. 4B, left panel), the binding of WRN to APE1 was detected. This interaction was not mediated by DNA as evidenced by the lack of effect of EtBr (left panel, lane 5). Furthermore, WRN did not bind to the BSA control (left panel, lane 2). When microtiter wells were coated with antibody that bound to WRN and then subsequently incubated with APE1 (Fig. 4B, right panel), a specific interaction between APE1 and WRN was also observed (right panel, lanes 3 and 4). Consistent with the ELISA, an interaction between WRN and APE1 was detected by dot blot (Fig. 4C, left panel). Interestingly, WRN did not bind to APE135, which lacks the N-terminal 35 amino acids of the full-length APE1 protein. Previous studies (42) indicate that this region of APE1 is involved in nuclear targeting, transcriptional regulation, and protein–protein interactions. A dot blot with truncated forms of WRN indicated that a C-terminal WRN fragment (GST-WRN949–1432), but not an N-terminal fragment or the helicase domain of WRN (data not shown), is sufficient for forming a stable complex with APE1 (Fig. 4C, right panel). In total, the results here indicate that the C terminus of WRN interacts with the N terminus of APE1. Notably, these results are consistent with the pull-down data in Fig. 4A.

WRN and APE1 also co-localize in U-2 osseosarcoma cells visualized by indirect immunofluorescence (Fig. 4D, WRN (red) and APE1 (green)). The staining for both proteins appears as a punctate pattern throughout the nucleus with over 25% foci
co-localizing for both proteins (Fig. 4D, yellow). In contrast, there is no co-localization (<2% foci) between APE1 and TRF2 (Fig. 4D, bottom panel). This finding indicates that, although WRN interacts with many different proteins at the same time, a considerable proportion of WRN and APE1 are present in the same punctate nucleoplasmic protein complexes in normal cells.

**A Protein-DNA Complex Mediates the Functional Interaction between WRN and APE1**—The mechanism by which APE1 inhibits WRN was explored further in the following experiments. As shown above (Fig. 3B), APE1 did not affect WRN helicase activity on forked duplex and 5'-recessed duplex substrates but did inhibit WRN helicase activity on BER intermediates. These data suggest that DNA binding of APE1 mediated inhibition of WRN helicase activity. In support of this finding, an APE1 DNA-binding mutant, H309N APE1, which lacks binding activity to incised F-nick site (41) but interacts with WRN (Fig. 4C), did not inhibit WRN unwinding of BER intermediates (Fig. 5, lanes 3–5). In addition, when APE1Δ35, which does not interact physically with WRN (Fig. 4C) and has comparatively weak DNA binding activity (Fig. 6, lanes 5–7 and 12–14), was included in the helicase reaction, WRN unwound the substrates to a similar extent as in the absence (Fig. 5, lane 8) or in the presence of a low concentration of APE1 (Fig. 5, lane 15). Thus, these results implied that the APE1-DNA complex formation regulates WRN helicase activity.

**Pol β Relieves APE1 Inhibition of WRN Unwinding Activity**—Previous studies (17) suggest that BER intermediates are sequentially passed from APE1 to pol β to facilitate the BER reaction, i.e. pol β recognizes the APE1-DNA complex, dissociates APE1 from the DNA, and “receives” the BER intermediate. Therefore, we were interested in testing whether the proposed protein switch affected WRN helicase function. As shown in Fig. 7, WRN helicase activity increased with increasing pol β in the presence of a constant amount of APE1 (lanes 4–6 and 11–13). These results suggested that protein switching by pol β
Fig. 3. APE1 inhibits WRN unwinding of BER intermediates. A, helicase reactions (10 μl) containing 1 nM DNA substrate, 4 nM WRN, and various concentrations of APE1 were performed as described under “Experimental Procedures.” Lanes 1 and 8, DNA substrate only. Lanes 2 and 9, DNA substrate and WRN (4 nM). Lanes 3–6 and 10–13, DNA substrate, WRN (4 nM), and increasing concentrations of APE1 (0.5, 1, 2, and 4 nM) as indicated. Lanes 7 and 14, heat-denatured substrates; % D, percent single-stranded product displaced. B, helicase reactions (10 μl) containing 1 nM DNA substrate, 4 nM WRN, and various concentrations of APE1 were performed as described under “Experimental Procedures.” Lane 1, DNA substrate only. Lane 2, DNA substrate and WRN (4 nM). Lanes 3–5, DNA substrate, WRN (4 nM), and increasing concentrations of APE1 (1, 2, and 4 nM) as indicated. Lane 6, heat-denatured substrates. % D, percent single-stranded product displaced. C, helicase reactions (10 μl) containing 1 nM DNA substrate and 4 nM WRN were performed as described under “Experimental Procedures.” Lanes 1 and 7, DNA substrate only. Lanes 2 and 8, DNA substrate and WRN (4 nM). Lanes 3–5 and 9–11, DNA substrate, WRN (4 nM), and increasing concentrations of APE1 (2, 4, and 8 nM) as indicated. Lanes 6 and 12, heat-denatured substrates.
FIG. 4. WRN physically interacts with APE1. A, GST-WRN<sub>949–1432</sub> pulls down APE1. GST (lane 2) or GST-WRN<sub>949–1432</sub> (lane 3) bound to glutathione beads was incubated with HeLa nuclear extracts (lane 1). APE1 bound to GST or GST-WRN was detected by Western blot analysis. Lane 4, purified APE1 (5 ng).

B, direct ELISA (left panel). Either BSA (bars 1 and 2) or purified APE1 (bars 3–5) was coated onto 96-well plates. After blocking with BSA, the wells were incubated with BSA (bars 1 and 3), purified WRN (bars 2 and 4), or ethidium bromide (10 μg/ml) plus

C, Blotted. Incubation

IB:poly-WRN

IB:mono-WRN

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is capable of bringing WRN to the BER intermediate site, allowing WRN to then catalyze the unwinding of the downstream oligonucleotide at a DNA strand break.

**DISCUSSION**

This study presented data that support a role for WRN in BER in mammalian cells. In particular, the results of this study showed that 1) WRN alone can unwind BER strand break intermediates, 2) WRN interacts specifically with APE1, and 3) APE1 inhibits WRN helicase activity, but this inhibition is alleviated by pol β. Thus, WRN is regulated in sequential reactions during the BER process via specific protein-DNA and protein-protein interactions.

Although WRN has previously been reported to inefficiently

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purified WRN (bar 5). Bound WRN was detected using anti-WRN antibodies and horseradish peroxidase-labeled secondary antibodies. The values represent the mean ± S.D. of at least three independent experiments performed in duplicate. Indirect ELISA is shown right panel. Either APE1 (bars 1 and 2) or anti-WRN antibodies (bars 3–5) were coated onto 96-well plates. The wells were incubated with BSA (bar 2) and WRN (bars 1 and 3–5). Subsequently, APE1 (bars 2–4) and BSA (bars 1 and 5) were added and incubated. After washing, interacting proteins were detected using anti-APE1 antibodies (bars 1–4) or anti-WRN antibodies (bar 5) followed by horseradish peroxidase-labeled secondary antibodies. The values represent the mean ± S.D. of at least three independent experiments. C, dot blot assay. 100 ng of APE1, APE1/Δ35, BSA, RPA, WRN, C-WRN, or GST as indicated were immobilized on a polyvinylidene difluoride membrane. The membranes were blocked for 2 h at 4 °C and incubated in the absence or presence of 400 ng/ml WRN as indicated at 4 °C overnight. After washing, the membranes were incubated with polyclonal (left panel) or monoclonal (right panel) anti-WRN antibodies. Western blotting was performed as described under “Experimental Procedures.” IB, immunoblotting. D, APE1 and WRN co-localize in the nucleus. U-2 osteosarcoma cells were processed for indirect immunofluorescence as described above and stained with rabbit anti-WRNp (1:200, red), anti-APE1 monoclonal antibody (1:100, green), and the DNA stain 4',6-diamidino-2-phenylindole dihydrochloride (blue). The bottom panel was stained with rabbit anti-APE1 (1:100, green) and anti-TRF2 monoclonal antibody (1:500, red). 0.1 μm Z-sections were obtained with an Axioplan 2i microscope (Carl Zeiss) and deconvolved using Openlab deconvolution software. Merge is the merged image of the three fluorescence channels examined from the same deconvolved Z-section (4',6-diamidino-2-phenylindole dihydrochloride was omitted for clarity). One representative cell from each set stained is shown. A section of each cell was enlarged (×3). ×630; bar, 5 μm.
unwind a nicked duplex (43, 44), we recently found that WRN unwinds a BER strand break intermediate produced following UDG and APE1 treatment of a uracil-containing oligonucleotide (25). However, the influence of UDG, APE1, and/or pol β on the unwinding reaction remained to be determined. Fig. 2 shows that WRN unwinds three distinct BER intermediates in the absence of BER proteins. In addition, WRN unwinds duplex DNA with a 1-nucleotide gap lacking the dRP residue. This result suggested that WRN recognizes a gap structure and unwinds DNA downstream from this entry site. When compared to 3′-overhang cognate substrates, WRN unwound strand break BER intermediates with a similar efficiency (Fig. 2, A and B). In addition, there was no significant difference between unwinding of the 5′-F and the 5′-AP downstream strand (Fig. 2, A and B).

WRN forms a stable complex with APE1 that can be detected in GST pull-down assays in HeLa NE, in ELISA assays, by immunofluorescence, and in dot blot assays with purified proteins. However, this complex does not form with N-terminally truncated APE1 missing amino acids 1–35 (APE1Δ35). Thus, the interaction between WRN and APE1 requires the N-terminal 35 amino acids of APE1. Although WRN interacts physi-
Fig. 7. APE1 inhibition of WRN unwinding is relieved by pol β. Unwinding reactions (10 μl) containing 1 nM DNA substrate, 4 nM WRN, and various concentrations of APE1 were performed as described under “Experimental Procedures.” Downstream primers were 3’ end-labeled with [γ-32P]dATP. Lanes 1 and 8, DNA substrate only. Lane 2, DNA substrate, APE1 (1 nM), and WRN (4 nM). Lane 9, DNA substrate and WRN (4 nM). Lanes 3 and 10, DNA substrate, WRN (4 nM), and APE1 (4 nM). Lanes 4–6 and 11–13, DNA substrate, WRN (4 nM), and increasing concentrations of pol β (1, 2, and 4 nM) as indicated. Lane 13, DNA substrate, WRN (4 nM), and pol β (4 nM). Lanes 7 and 14, heat-denatured substrates. Percent of displacement relative to that of lanes 2 and 9 was calculated. Values represent the mean ± S.E. of two independent experiments.

Fig. 8. A scheme of WRN regulation during long patch BER. A DNA base lesion (U:G mispair) is recognized by UDG, and UDG bound to its product is displaced by APE1. WRN does not recognize the APE1-DNA complex, whereas pol β recognizes and displaces APE1. At this step, WRN recognizes the pol β-DNA complex and stimulates pol β DNA strand displacement synthesis. Proteins are indicated by a diamond for UDG, a hexagon for APE1, a oval for pol β, and a triangle for WRN.
cally with APE1, WRN has no discernable effect on APE1 nuclease activities in vitro (data not shown). Thus, we propose that the APE1-WRN interaction simply brings WRN to sites where DNA lesions are being actively repaired by BER. Whereas wild-type APE1 forms a tight complex with AP substrate, APE1Δ35 does not bind stably to the AP site containing DNA as determined by electrophoretic mobility shift assays, even though it has an efficient AP endonuclease activity, identifying a novel role for the N terminus of APE1 in mediating the APE1-DNA complex stability.

Interestingly, earlier studies (45) show that APE1 inhibits undesired DNA ligase I activity on F-nick substrates. Therefore, APE1 probably limits access to the nick by blocking the binding of DNA ligase I, suggesting a tight interaction between APE1 and its reaction product. Similar results were observed here for WRN and APE1 in the presence of APE1 DNA substrates (Fig. 3, A and B). A tight complex between APE1 and its reaction product might perform a biologically relevant function by protecting cleaved abasic sites from religation or unwinding before repair is completed by downstream enzymes in the BER pathway.

Structural studies of BER enzymes in complex with their DNA substrate or product were recently completed. These studies led to a proposal of coordinated product-substrate hands-off by enzymes in the BER pathways (17). When both pol β and APE1 are incubated with AP-containing DNA and the reaction is analyzed by electrophoretic mobility shift assays, protein DNA complexes are detected that include a nicked DNA substrate and pol β (19), suggesting that these two proteins are coordinated in sequential reactions. Because APE1 is in excess over pol β in vivo (38), the proposed hand-off mechanism might facilitate efficient AP site repair by increasing the probability that pol β binds to AP-DNA after the APE1-AP-DNA complex has been formed. These ideas are consistent with a fast and specific relay of DNA substrates from one enzyme to the next in the BER pathway. This efficient coordination is also consistent with results presented here, indicating that WRN helicase is not inhibited by APE1 in the presence of pol β. Based on earlier studies demonstrating a functional interaction between WRN and pol β (25), we propose that pol β may recruit WRN to nicked BER intermediates to facilitate the unwinding of DNA downstream of the break.

A model summarizing our results is presented in Fig. 8. APE1 recognizes and binds to an AP site and incises the phosphodiester backbone 5’ to the AP lesion. APE1 remains associated with the nicked DNA product of this reaction, thereby preventing promiscuous unwinding of the nick by WRN (Fig. 3). By transiently sequestering the BER intermediate, APE1 plays an important role in regulating progression of the BER pathway. APE1 can then be replaced by pol β after which BER branches into one of two subpathways, short patch or long patch BER. In short patch BER, pol β inserts a single nucleotide. Alternatively, APE1 and pol β interact with WRN and the synthesis and unwinding of 2-10 nucleotides downstream of the nicked AP site as part of long patch BER occur in a coordinated manner (Fig. 8).

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