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The N-terminal domain of the large subunit of human replication protein A binds to Werner syndrome protein and stimulates helicase activity

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Abstract

Werner syndrome (WS) is a recessive inherited human disease characterized by the early onset of aging. The gene mutated in WS encodes a DNA helicase that unwinds the double helical structure of DNA in the 3′→5′ direction as well as a 3′→5′ exonuclease. Our previous studies indicated that the activity of Werner syndrome helicase (WRN) could be stimulated by human replication protein A (hRPA), a heterotrimeric single-stranded DNA binding protein. We now localize the interaction between WRN and hRPA by measuring the stimulation of helicase activity and the binding of WRN by hRPA and its derivatives. The large subunit of hRPA (hRPA70) stimulates WRN helicase to the same extent as the hRPA heterotrimer, whereas the dimer of the two smaller subunits (hRPA 32·14) does not stimulate. By examining hRPA70 mutants with progressive deletions from either the C- or N-terminus, we found that the domain responsible for stimulation lies in the N-terminal half of the protein. By using enzyme-linked immunosorbent assay (ELISA) to examine physical interaction between WRN and the same deletion mutants, we found that the WRN-binding motif is located within amino acids 100–300 and overlaps with the single-stranded DNA binding domain (amino acids 150–450). We suggest that hRPA, by engaging in both protein–protein and protein–DNA interactions, facilitates unwinding events catalyzed by WRN helicase during DNA synthetic processes. These data should help further elucidation of the molecular mechanisms of genetic instability and premature aging phenotypes manifested by WS.

Keywords: Werner syndrome; DNA helicase; Replication protein A; Premature aging; Genetic instability

1. Introduction

Werner syndrome (WS) is an autosomal recessive genetic disorder that is manifested by the premature onset of many features of aging (Brosh and Bohr, 2002; Lebel, 2001; Shen and Loeb, 2000). Individuals with this disease often develop age-related pathologies including atherosclerosis and malignant neoplasms in early adulthood. The types of cancers associated with WS are unusual and include sarcomas, meningiomas and melanomas. Cultured WS cells exhibit a shortened life span and a prolonged S-phase. Importantly, WS cells display genetic instability, manifested by chromosomal translocations (Salk et al., 1981) and increased mutation rates mainly associated with extensive DNA deletions (Fukuchi et al., 1989). Disease-associated mutations in the WS gene, Werner syndrome helicase (WRN), invariably encode deletions or stop codons predicted to result in truncations of WRN protein, with loss of catalytic activity and deletion of a potential nuclear localization signal at the C-terminus (Goto et al., 1997; Matsumoto et al., 1997; Oshima et al., 1996; Yu et al., 1996). Therefore, loss of function of WRN is expected in homozygous individuals (Matsumoto et al., 1997). While WS is uncommon, the incidence of heterozygotes in the population may be as frequent as one in four hundred (Schellenberg et al., 2001). A second sponta-

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neous mutation in the other allele could be associated with an increased incidence of cancers associated with WS.

WRN locates on chromosome eight at 8p12 (Goto et al., 1992) and encodes a protein containing 1432 amino acids (Yu et al., 1996). A conserved DExH DNA helicase domain at the center of the gene places WRN in the *Escherichia coli* RecQ helicase family. On the basis of extensive sequence alignments, a second activity homologous with the proof-reading exonuclease of *E. coli* DNA polymerase I and *E. coli* RNase D was predicted to reside near the N-terminus of the protein (Moser et al., 1997; Mushegian et al., 1997). Consistent with these predictions, WRN has been demonstrated in vitro to be a $3'\rightarrow5'$ DNA helicase (Gray et al., 1997; Suzuki et al., 1997) and a $3'\rightarrow5'$ dsDNA exonuclease (Huang et al., 1998; Shen et al., 1998a). In addition to WRN helicase, the RecQ family includes *E. coli* RecQ (Nakayama et al., 1984), *Saccharomyces cerevisiae* Sgs1 (Gangloff et al., 1994; Watt et al., 1995), *S. pombe* Rqh1 (Murray et al., 1997; Stewart et al., 1997), human RecQL (Puranam and Blackshear, 1994; Seki et al., 1994), human Bloom syndrome helicase (BLM) (Ellis et al., 1995), human Rothmund–Thomson syndrome helicase (RecQL4) (Kitao et al., 1999) and human RecQL5 (Kitao et al., 1998). Notably, of all known RecQ helicases, WRN is the only one encoding both helicase and exonuclease activities. However, the role of WRN in DNA metabolic process remains to be established.

Human RPA (human single-stranded DNA binding protein) is a heterotrimer that has been reported to interact with several proteins in different DNA metabolic pathways (Wold, 1997), including the replication proteins SV40 large T-antigen and DNA polymerase α (Dornreiter et al., 1992), the repair proteins XPA and XPG (Xeroderma pigmentosum complementing groups A and G) (He et al., 1995; Matsuda et al., 1995), the recombination proteins Rad51 and Rad52 (Gasior et al., 1998; New et al., 1998), the transcription factors GAL4 and VP16, and the cell cycle regulating protein p53 (He et al., 1993; Li and Botchan, 1993). In contrast, known protein–protein interactions of WRN include hRPA (Brosh et al., 1999; Shen et al., 1998b), p53 (Blander et al., 1999; Spillare et al., 1999), topoisomerase I (Lebel et al., 1999), PCNA (Lebel et al., 1999), Ku 86/70 (Cooper et al., 2000), DNA polymerase δ (Kamath-Loeb et al., 2000), DNA-PK (Karmakar et al., 2002), BLM (von Kobbe et al., 2002), TRF2 (Opresko et al., 2002) and FEN-1 (Brosh et al., 2001).

While a number of proteins have been shown to bind to WRN, only a few has been demonstrated to significantly effect catalytic activity. Of these reported WRN-interacting proteins, hRPA and TRF2 have been demonstrated that can functionally stimulate the helicase activity of WRN. In this paper we report that the N-terminal region of the hRPA large subunit (hRPA70) mediates protein–protein association with WRN and stimulation of WRN helicase activity.

2. Materials and methods

2.1. Proteins and antibodies

The mutant forms of hRPA used in this study have been described previously (Braun et al., 1997) and are illustrated in Fig. 1. Their nomenclature is as follows. Single subunits carrying deletions are indicated by the name of the subunit followed by description of the deletion; for example, hRPA70ΔC442 is the hRPA70 subunit containing a C-terminal deletion from amino acid 442. Complexes of multiple subunits are indicated by hRPA followed by the numeric designation of the mutant subunit and a description of the deletion. Deletions are indicated by ΔNXXX or ΔCXXX where XXX refers to residues deleted either from the N- or C-terminus, respectively. Recombinant hexahistidine-tagged WRN protein was expressed in insect cells by using a baculovirus expression system and purified by sequential chromatographic steps, as described previously (Shen et al., 1998a). Preparations of rabbit polyclonal antisera against wild-type WRN protein and hRPA were also described previously (Shen et al., 1998a; Braun et al., 1997; Dornreiter et al., 1992).

2.2. Helicase activity assay

The strand displacement assay for measurement of WRN helicase activity in the presence of hRPA has been described (Shen et al., 1998b). Briefly, in a typical reaction (10 μl final volume) 10 fmol of the 5′-^{32}P-labeled 42-mer/ssM13mp2 partial duplex substrate was treated with 12.5 fmol of WRN protein in the absence or presence of the indicated concentrations of different hRPA constructs. The mixture was incubated at 37 °C for 10 min in buffer containing 40 mM Tris–HCl (pH 7.4), 4 mM $MgCl_2$, 5 mM DTT, 100 μg/ml BSA and 1 mM ATP. Reactions were terminated by addition of 2 μl of 40% glycerol, 50 mM EDTA, 2% SDS, 3% bromophenol blue and 3% xylene cyanol. Products were resolved by electrophoresis through a 12% polyacrylamide gel in 1 × TBE buffer (90 mM Tris base, 90 mM boric acid, 1 mM EDTA) at 4 °C for 2 h (20 V/cm). The gel was vacuum dried and the extent of DNA displacement was visualized by autoradiography or quantified by PhosphorImager (Molecular Dynamics) analysis.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Binding of purified recombinant hRPA and WRN protein was examined by ELISA as described previously (Braun et al., 1997). Ninety six-well microtiter plates

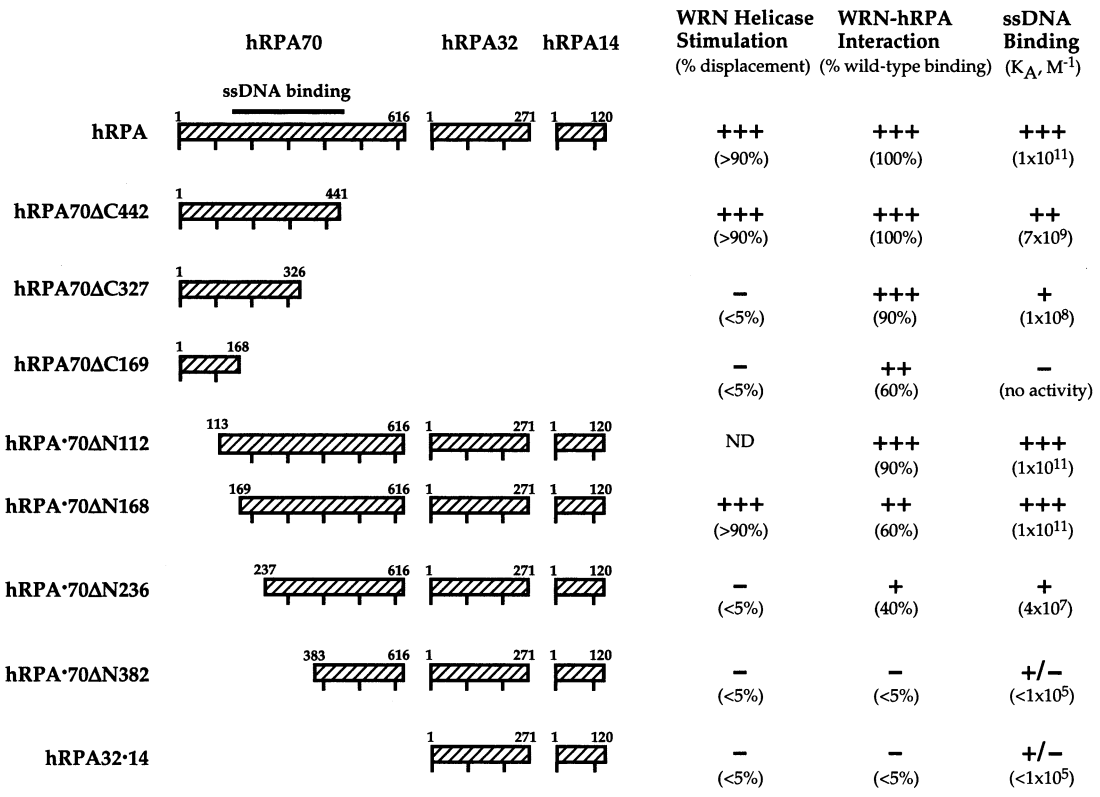


Fig. 1. Schematic representation and activities of wild-type and mutant hRPA proteins. Wild-type hRPA and the derivatives used in this study are diagrammed at the left. The relative activities of these proteins, i.e. WRN helicase stimulation, WRN–hRPA interaction assessed by ELISA, and ssDNA binding (Gomes and Wold, 1996; Sibenaller et al., 1998; Walther et al., 1999) are presented at the right. WRN helicase stimulation: (+++), >90% displacement of a 42-mer from partially duplex ssM13 catalyzed by WRN in the presence of 230 nM wild-type or mutant hRPA; (−), <5% displacement observed in the presence of 230 nM wild-type or mutant hRPA; ND, not determined. WRN–hRPA interaction: (+++), extent of WRN-binding by wild-type hRPA; (++), about 60% of wild-type binding; (+), about 40% of wild-type binding; (−), <5% of wild-type binding. ssDNA binding activity: (+++), wild type binding, $K_A = 1 \times 10^{11}$ M^{-1}; (++), binding reduced by about an order of magnitude; (+), binding reduced by about three orders of magnitude; (+/−), binding detectable but reduced by more than six orders of magnitude; (−), no detectable binding.

were coated with either 10 pmol of wild-type/mutant hRPA proteins or 1.25 pmol (0.2 μg) of WRN protein for 1 h at 25 °C. The plates were washed three times with phosphate buffered saline (PBS) containing 0.2% Tween 20. The plates were then blocked with 5% non-fat dry milk (NFDM) in PBS for 10 min at 25 °C. The wells were washed and incubated with increasing amounts of the indicated second protein (either WRN or truncated hRPA) in PBS/5% NFDM for 1 h at 25 °C. The plates were washed and incubated with either anti-WRN or anti-hRPA polyclonal antiserum in PBS/5% NFDM for 30 min at 25 °C. Bound antibody was detected by incubation with goat *anti*-rabbit IgG peroxidase conjugate (Sigma) and 0.8 mg/ml *O*-phenylenediamine in 0.05 M phosphate–citrate buffer/0.03% sodium perborate at 25 °C. Hydrolysis of *O*-phenylenediamine to an orange chromophore was monitored at 405 nm; optical density was quantified multiple times between 20 and 60 min to ensure linearity of the assay and the consistency of individual readings. Non-specific interactions of hRPA and WRN were monitored by using a BSA control; these values were routinely subtracted from the final measurements.

3. Results

3.1. The N-terminal region of hRPA70 is sufficient for stimulation of WRN helicase activity

The WRN helicase, by itself, is only able to unwind a short stretch of partially duplex DNA. We have previously shown that hRPA heterotrimer enables WRN helicase to efficiently unwind a partially duplex DNA consisting of a 42-mer hybridized to ssM13 DNA. WRN alone cannot catalyze this reaction (Shen et al., 1998b). Since human RPA contains three subunits—hRPA70, hRPA32, and hRPA14 (Wold, 1997)—we first identified the subunit(s) responsible for stimulating WRN helicase activity, and then mapped the domain(s) that functionally interacts with WRN. To address these questions, we measured the extent of WRN-catalyzed

unwinding of the 42-mer partial DNA duplex (see 40 and Section 2) in the presence of various hRPA subunit(s) or truncated hRPA proteins.

WRN (1.25 nM) cannot unwind the 42-mer/ssM13 partial duplex (1 nM) during a 10 min incubation at 37 °C (Shen et al., 1998b). However, as shown in Fig. 2A, WRN can efficiently displace the 42-mer (>90% displacement of total substrate) in the presence of 170 or 230 nM of hRPA heterotrimer. An equivalent amount of the hRPA32·14 dimer lacking the hRPA70 subunit is

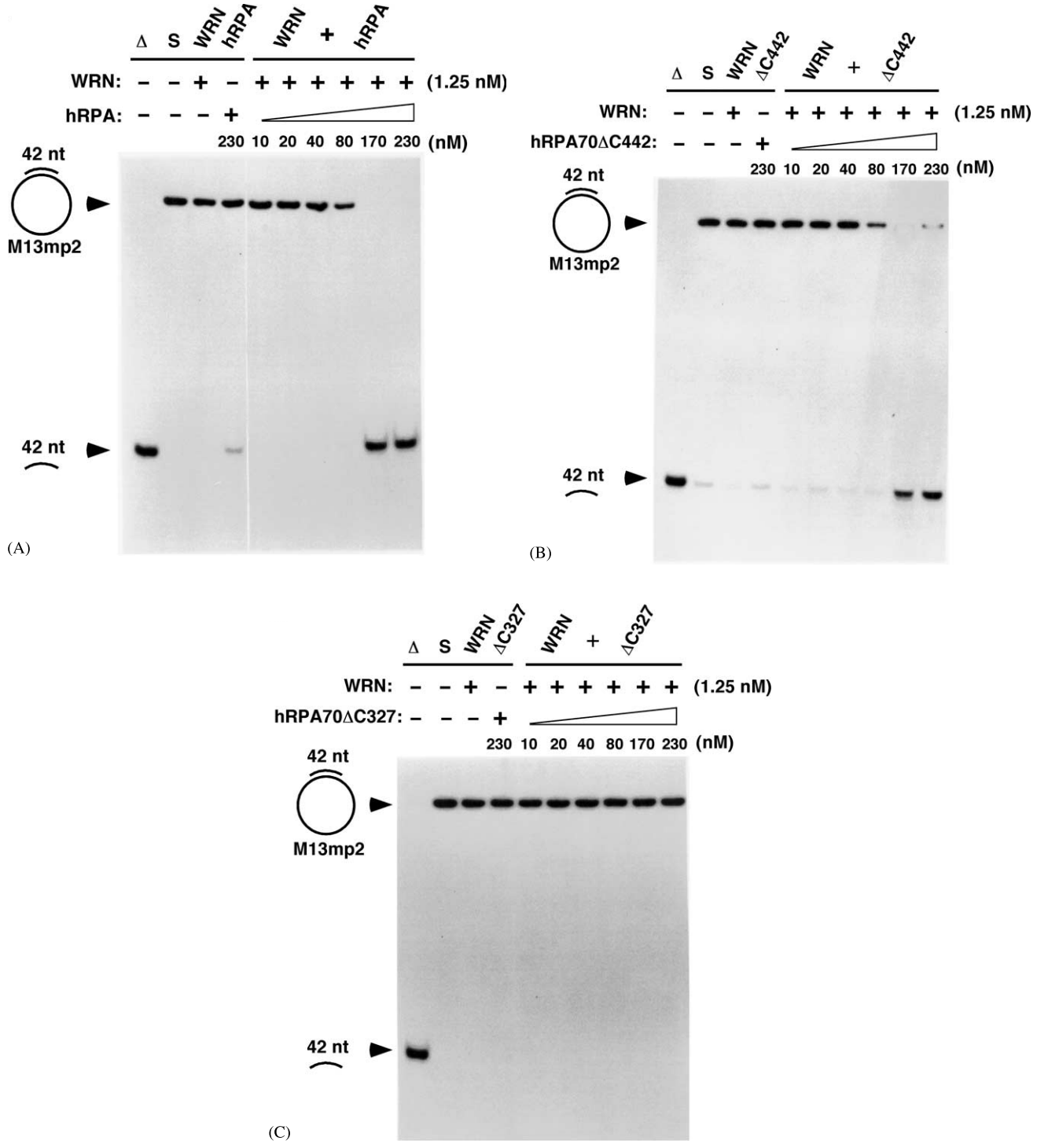


Fig. 2. Stimulation of WRN helicase activity by hRPA mutants. The partially duplex substrate 5′-^{32}P-42-mer/ssM13mp2 (1 nM) was incubated with WRN (1.25 nM) at 37 °C for 10 min in the absence or presence of increasing amounts of hRPA or a mutant derivative. Reaction products were resolved by 12% polyacrylamide gel electrophoresis and visualized by autoradiography. S, substrate; Δ, boiled substrate. (A) Wild-type hRPA heterotrimer. (B) hRPA70ΔC442. (C) hRPA70ΔC327.

unable to stimulate WRN helicase activity (< 5% displacement, Fig. 1, first data column), suggesting that hRPA70 is responsible for the stimulation.

In order to map the hRPA70 domain that functionally interacts with WRN, we employed a series of N- and C-terminal truncation mutants (Fig. 1). The C-terminal deletion mutants do not contain the 32 and 14 kDa subunits. As demonstrated in Fig. 2B, the mutant hRPA70ΔC442 lacking the C-terminal 175 residues (amino acids 442–616) stimulated activity to the same extent as wild-type hRPA, i.e. > 90% displacement in reactions containing 170 or 230 nM. However, the mutant hRPA70ΔC327 lacking the C-terminal 290 residues (amino acids 327–616) failed to detectably stimulate activity (Fig. 2C), even at a 10 times higher concentration (2.3 μM, data not shown). This sharp discrepancy between hRPA70ΔC442 and hRPA70ΔC327 is likely due to a 70-fold reduction in the ssDNA binding ability of ΔC327 relative to that of ΔC442 (Fig. 1). The decrease in the K_A for ssDNA may result from a more extensive deletion of the DNA binding domain (amino acids 150–450) in ΔC327 vs. ΔC442. If so, our observations suggest that the ssDNA binding activity of hRPA plays a role in stimulating DNA unwinding by WRN. Consistent with this prediction, the mutant hRPA70ΔC169 that lacks most of the DNA binding domain was unable to stimulate activity (Fig. 1). Thus it appears that the region of hRPA70 that functionally interacts with WRN lies between amino acids 1 and 441.

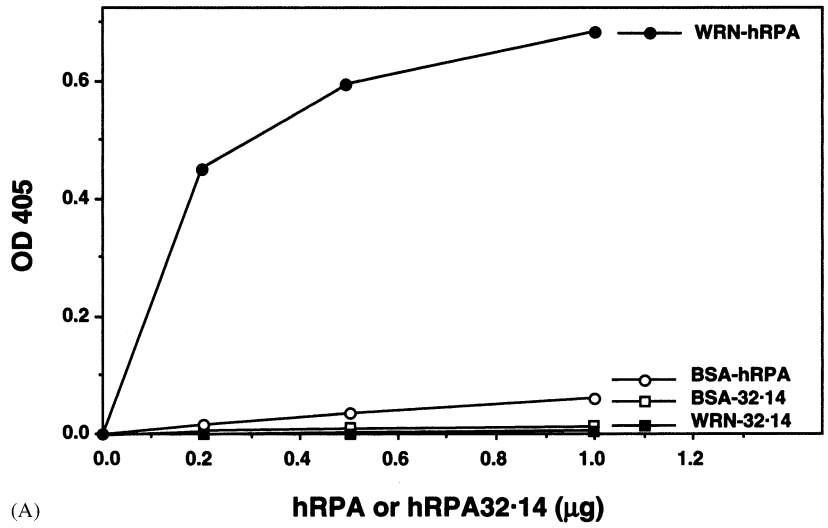
To determine whether the region of hRPA70 upstream of the ssDNA binding domain is necessary for WRN helicase stimulation, we examined N-terminal deletion mutants. The mutants consisted of N-terminal truncations of hRPA70 that were reconstituted with the 32 and 14 kDa subunits to form various hRPA heterotrimers. As shown in Fig. 1, hRPA·70ΔN168 (a mutant heterotrimer lacking residues 1–168 of hRPA70) stimulated WRN helicase activity as efficiently as the wild type heterotrimer (> 90% displacement of total substrate at a protein concentration of 170 or 230 nM). However, two additional mutants with more extensive deletions that span the ssDNA binding domain, hRPA·70ΔN236 and hRPA·70ΔN382, bound weakly to DNA and did not confer stimulation (Fig. 1). These results indicate that the putative WRN-interacting domain on hRPA70 encompasses amino acids 169–441 and overlaps with the ssDNA binding domain (amino acids 150–450).

3.2. The N-terminal region of hRPA70 interacts with WRN

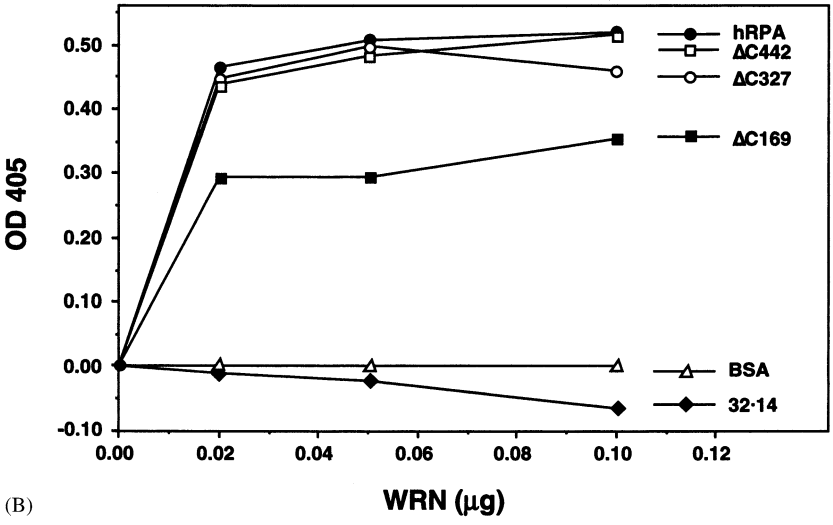
Our results with truncated hRPA proteins suggest that WRN helicase activity can be stimulated either by a direct association of hRPA with WRN, or indirectly, by the ability of hRPA to bind ssDNA and prevent re-annealing of the displaced DNA strand. To distinguish these possibilities, we employed ELISA to examine the physical interaction of hRPA with WRN in the absence of DNA. Purified recombinant WRN helicase was fixed to a 96-well microtiter plate and incubated with either the hRPA heterotrimer or the 32·14 kDa dimer, followed by anti-RPA antiserum and a secondary enzyme-coupled antibody as described in Section 2. As shown in Fig. 3A, an enzymatic signal (measured by a spectrophotometric assay at 405 nm) was detected only when WRN was incubated with the hRPA heterotrimer, suggesting a direct protein–protein association. The interaction of the hRPA trimer with WRN was specific, since a BSA control showed no association with hRPA. In addition, no physical interaction was detected between WRN and the 32·14 kDa dimer. These results are consistent with those of the functional assays (Fig. 1), and suggest that hRPA70 is the only subunit that directly interacts with WRN, even in the absence of DNA.

We further investigated the putative interacting motifs within hRPA70 by examining the C-terminal deletion mutants described above. In this experiment, wild-type or mutant hRPA proteins were fixed to the ELISA plate and then incubated with various concentrations of WRN. Mutants with C-terminal deletions of 175 residues (hRPA70ΔC442) or 290 residues (hRPA70ΔC327) bound to WRN as tightly as wild-type hRPA (> 90% of the binding of wild-type hRPA at 0.1 μg of WRN, Fig. 3B and Fig. 1). In contrast, the extreme C-terminal deletion mutant hRPA70ΔC169 retained only 60% of wild-type binding. These data support our conclusions from the functional interaction assays and indicate that the WRN-binding motif is at the N-terminus of the 70 kDa hRPA subunit (amino acids 1–326 in this experiment) and overlaps with the ssDNA binding domain (amino acids 150–450).

To more precisely delineate the contact region, we examined mutant heterotrimers containing different N-terminal truncations of hRPA70 (Fig. 1). As presented in Fig. 3C, mutants with progressive deletions of N-terminal amino acids (i.e. hRPA·70ΔN112,·70ΔN168,·70ΔN236 and·70ΔN382) exhibited progressive loss of WRN-binding ability. The binding observed for these mutants relative to wild-type hRPA (defined as 100% at 0.1 μg of WRN) was ΔN112 (90%) > ΔN168 (60%) > ΔN236 (40%) > ΔN382 (3%) > BSA (0%). These data again suggest that the WRN-binding motif lies within the N-terminal region of hRPA70, overlapping with the ssDNA binding domain. In summary, all of the results shown in Fig. 3 strongly indicate that the WRN-interacting region of wild-type hRPA heterotrimer is located in hRPA70 approximately between amino acids 100 and 300.



(A)



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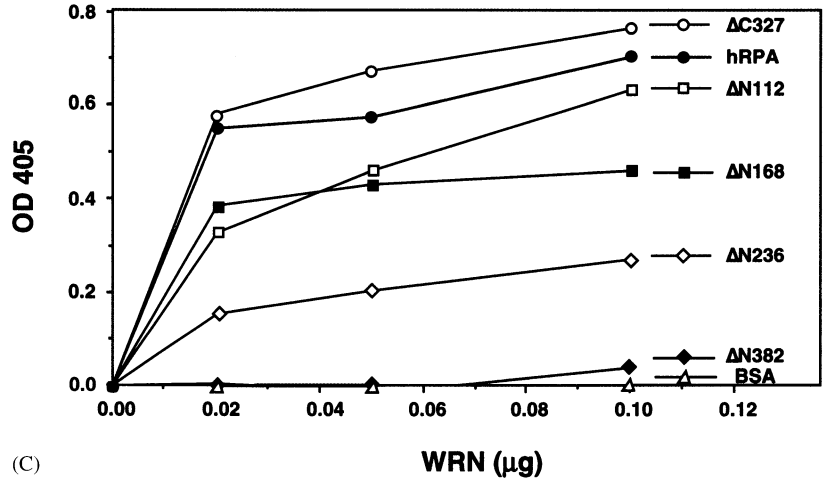

(C)

Fig. 3. WRN–hRPA interaction observed by ELISA. (A) WRN (0.2 μg) or BSA (0.2 μg) was immobilized on microtiter plates. Increasing amounts (μg) of hRPA or hRPA32·14 dimer were added, and after incubation, the plates were washed. The bound proteins were detected by polyclonal anti-hRPA antibody conjugated with anti-rabbit IgG peroxidase under conditions that yield optical density at 405 nm. The OD_{405} was plotted against the amount of hRPA or hRPA32·14. (B) and (C) 10 pmol of wild-type hRPA, or one of its derivatives (mainly C-terminally truncated proteins of hRPA70), or BSA was immobilized on microtiter plates and increasing amounts of WRN (μg) were added, and after incubation, the plates were washed. Bound proteins were detected by methodology similar to described in (A) by using polyclonal anti-WRN antibody. The OD_{405} was plotted against the amount of WRN. In all cases multiple trials were carried out and a single representative experiment is shown.

4. Discussion

We have purified the Werner syndrome protein (WRN) and demonstrated that WRN possesses both $3' \rightarrow 5'$ helicase activity (Shen et al., 1998b) and a physically distinct $3' \rightarrow 5'$ exonuclease avtivity (Shen et al., 1998a). We also observed that WRN helicase activity is stimulated by human RPA. The stimulation conferred by hRPA was greater than that observed for SSBs from *E. coli* and bacteriophage T4 (Shen et al., 1998b). Brosh et al. (1999) confirmed and extended our initial findings, showing that, in the presence of hRPA, WRN can unwind stretches of double-stranded DNA as long as 849 nucleotides. These authors also provided evidence for direct, protein–protein interaction by demonstrating co-immunoprecipitation of WRN and hRPA. Here, we confirm the physical interaction by ELISA and show that the WRN-interacting domain on hRPA lies in the N-terminal half of the large 70 kDa subunit (hRPA70). Moreover, the N-terminal domain of hRPA70 is sufficient to stimulate WRN helicase activity. Our results suggest that hRPA may engage not only in functional interaction, but also in direct association, with WRN and play an important role in WRN-mediated, multiple metabolic pathways involving DNA structure resolution.

In these studies, we first quantified the stimulation of WRN helicase activity conferred by different truncated hRPA proteins, and observed that the helicase-enhancing activity is localized between amino acids 169 and 441 in the N-terminus of the large subunit (Fig. 1). More detailed mapping by ELISA revealed that the region of physical interaction in hRPA70 lies approximately between amino acids 100 and 300 (Figs. 1 and 3). Interestingly, this putative WRN-interacting region overlaps with the ssDNA-binding domain that resides between amino acids 150 and 450. The overlap suggests that the ssDNA binding activity of hRPA is involved in stimulating WRN helicase to unwind long DNA duplexes.

The mutant hRPA proteins we examined differ greatly in K_A values for binding to ssDNA, due to varying deletions of the ssDNA binding domain ((Gomes and Wold, 1996; Sibenaller et al., 1998; Walther et al., 1999) and Fig. 1). Reduction in ssDNA binding affinity is associated with reduction in WRN helicase stimulation (Fig. 1). For example, the C-terminal deletion mutant hRPA70ΔC327, which retained 90% of wild-type WRN-binding activity but carries a truncated ssDNA binding domain that reduces the K_A by three orders of magnitude, did not stimulate WRN helicase activity. In contrast, the N-terminal deletion mutant hRPA·70ΔN168, which retains only 60% of wild-type WRN-binding activity but contains an intact ssDNA binding domain, stimulated WRN helicase to the same extent as wild-type hRPA. Moreover, the deleted portion of hRPA·70ΔN168, hRPA70ΔC169, which likewise retains 60% of wild-type WRN-binding activity but has no ssDNA binding activity, did not stimulate WRN helicase.

In *E. coli*, the WRN homolog RecQ plays a role in the RecF recombinational pathway (Kowalczykowski et al., 1994) and functions as a suppressor of illegitimate recombination (Hanada et al., 1997). In vitro, *E. coli* RecQ helicase can be stimulated by SSB (Umezu and Nakayama, 1993), consistent with our observations on WRN and hRPA. Moreover, *E. coli* Rec Q, along with SSB and RecA, can unwind several joint molecules, consistent with functions in initiation of homologous recombination and disruption of aberrant recombination (Harmon and Kowalczykowski, 1998). In *S. cerevisiae*, *sgs1* mutants exhibit hyperrecombination (Watt et al., 1996, 1995), and the increased recombination can be suppressed by human WRN or BLM (Yamagata et al., 1998). Interestingly, homologous recombination is also elevated in some WS cells (Cheng et al., 1990; Prince et al., 2001). Other studies suggest that *E. coli* RecQ is involved in re-initiation of replication fork arrest (Courcelle and Hanawalt, 1999). Notably, WRN has been shown to co-localize with hRPA and Rad51 upon replication fork arrest (Constantinou et al., 2000; Sakamoto et al., 2001). Thus, WRN–hRPA complex may function in recombination-mediated DNA synthesis or in recombinational repair induced by replication fork arrest (Shen and Loeb, 2000). Alternatively, the *Xenopus* homolog of WRN, FFA-1 (replication foci forming activity 1) (Yan et al., 1998), binds to chromatin in the initial stage of assembling RPA-containing replication origin complexes, suggesting a possible role of WRN in replication.

On the basis of our data, we infer that both protein–protein and protein–DNA interactions are likely required for hRPA to stimulate WRN helicase in unwinding extensive stretches of duplex DNA. We propose a model, illustrated in Fig. 4, that accounts for the interaction of hRPA with both WRN and ssDNA and for the stimulation of WRN helicase activity. We suggest that hRPA first binds to ssDNA and that the ssDNA-bound hRPA then recruits WRN to the ss/ds DNA junction through protein–protein interaction. Alternatively, WRN and hRPA could first form a complex and then bind to the ss/ds DNA junction. As WRN translocates $3' \rightarrow 5'$ along the DNA, it transfers the associated hRPA onto the displaced DNA strand. As translocation proceeds, WRN transfers more hRPAs on to the displaced ssDNA to prevent re-annealing of the unwound strand. Structural studies have shown that the high affinity DNA binding domain in the central region of hRPA70 interacts with eight nucleotides of DNA (Bochkarev et al., 1997). Furthermore, hRPA can form stable complexes with oligonucleotides only 10–12 nt long (Kim et al., 1994). Therefore, hRPA, by means

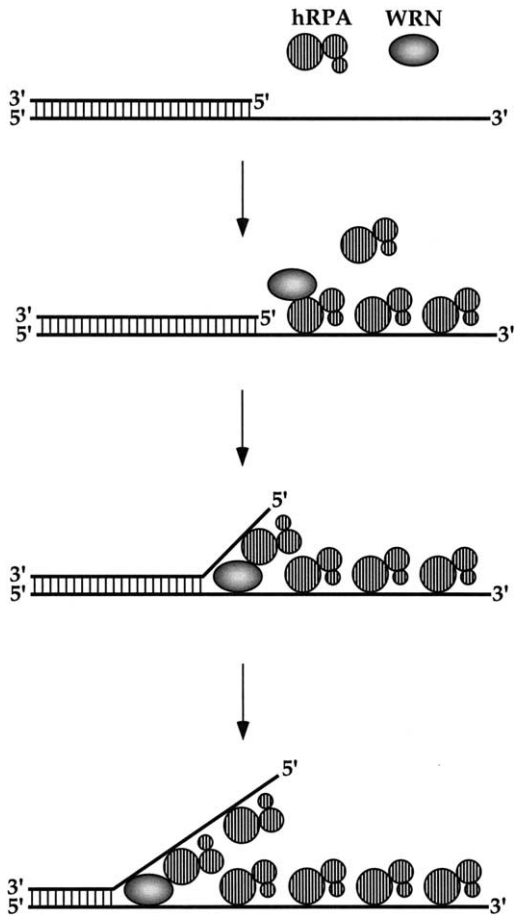

Fig. 4. A schematic model for hRPA-facilitated translocation of WRN helicase. Single-stranded DNA generated in the processes of DNA metabolism is first bound and stabilized by hRPA. Through specific WRN–hRPA interaction, WRN is then recruited by hRPA to the single/double-stranded DNA junction, prior to initiation of WRN-catalyzed unwinding. When translocation begins, WRN moves 3′→5′ along the bound DNA strand and leaves behind the displaced single-stranded DNA, which is bound and stabilized immediately by the transiently WRN-associated hRPA. The hRPA-bound single-stranded DNA is thereby prevented from reannealing, thus allowing WRN to move forward more efficiently. hRPA thus facilitates processive translocation of WRN through both the avidity for WRN and the nature of its single-stranded DNA binding activity.

of its interactions with WRN and ssDNA, facilitates the translocation of WRN helicase during DNA unwinding. This model is similar to that proposed by Boehmer in studies of DNA helicase UL-9 and ssDNA-binding protein ICP-8 from human type I herpes simplex virus (Boehmer, 1998).

WRN-deficient cells exhibit genetic instability manifested at the cytogenetic level by variegated translocational mosaicism (Salk et al., 1981), and at the molecular genetic level by extensive deletions (Fukuchi et al., 1989). Interestingly, RPA mutants in *S. cerevisiae* also exhibit genomic instability characterized by multiple DNA deletions (Chen et al., 1998). These findings may indicate that WRN and hRPA function synergistically in maintaining the integrity of genomic DNA. The incidence of WRN heterozygotes in the human population may be as great as one in four hundred (Schellenberg et al., 2001). A second mutation in WRN in somatic cells could result in an increased incidence of cancers, specifically those associated with WS. The data presented here indicates that a mutation in hRPA may also diminish WRN function. The mutations in these genes may act synergistically to increase mutagenesis and perhaps accelerate tumorigenesis.

Acknowledgements

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