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Mini Review

Werner syndrome protein: Functions in the response to DNA damage and replication stress in S-phase

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Abstract

Werner syndrome (WS) is an excellent model system for the study of human aging. WRN, a nuclear protein mutated in WS, plays multiple roles in DNA metabolism. Our understanding about the metabolic regulation and function of this RecQ helicase has advanced greatly during the past decade, largely due to the availability of purified WRN protein, WRN knockdown cells, and WRN knockout mice. Recent biochemical and genetic studies indicate that WRN plays significant roles in DNA replication, DNA repair, and telomere maintenance. Interestingly, many WRN functions require handling of DNA ends during S-phase, and evidence suggests that WRN plays both upstream and downstream roles in the response to DNA damage. Future research should focus on the mechanism(s) of WRN in the regulation of the various DNA metabolism pathways and development of therapeutic approaches to treat premature aging syndromes such as WS.
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1. Werner syndrome (WS) as a model system to study normal aging

Studies of progeroid syndromes have advanced our understanding of how defects in cellular responses to DNA damage contribute to the aging process. Werner syndrome (WS), the best studied progeroid syndrome, is an autosomal recessive disorder characterized by inactivating mutations in *WRN*, the gene encoding WS protein (WRN). WRN belongs to the conserved RecQ family of DNA helicases. Mutations in other RecQ family members in humans cause Bloom syndrome and Rothmund–Thomson syndrome, both of which also exhibit features of premature aging (for comprehensive reviews on RecQ helicases, see (Hickson, 2003; Opresko et al., 2004a)). WS is characterized by predisposition to aging-related pathologies, the most prevailing of which is bilateral ocular cataracts (Huang et al., 2006). In addition, type II diabetes, arteriosclerosis, osteoporosis, and tumors of mesenchymal origin are commonly observed in WS patients (Martin and Oshima, 2000). Patients begin to show signs of accelerated aging after puberty. Based on the updated International Registry of Werner syndrome (www.wernersyndrome.org), the average age of death from WS is 54 years, usually as a result of cancer and arteriosclerosis (Huang et al., 2006). Noticeably, a splicing mutation that results in the deletion of exon 26 is found in 80% of WS patients of Japanese origin (Moser et al., 1999). Other *WRN* mutations include nonsense and frameshift mutations, some of which generate C-terminally truncated proteins that lack a nuclear localization signal and are degraded in the cytoplasm. Interestingly, 20% of the patients diagnosed with WS lack *WRN* mutations and are classified as atypical WS patients. Among atypical WS patients, 15% carry missense mutations in the Lamin A gene (Chen et al., 2003). Strikingly, a rare childhood syndrome of premature aging, Hutchinson–Gilford syndrome, is also associated with mutations in Lamin A and is characterized by genomic instability (Liu et al., 2005). Although patients with atypical WS

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and Lamin A mutations do not develop cataracts or diabetes, evidence from these two representative progeria syndromes lend support to the hypothesis that genomic instability is positively associated with the process of normal aging. Intriguingly, the pattern of altered gene expression in fibroblasts derived from WS patients is reminiscent of the array of changes in cells from naturally aged control individuals (Kyng et al., 2003). Moreover, the aging phenotypes observed in WS have been reproduced in a *WRN* knockout mouse model in a short telomere background (Chang et al., 2004; Du et al., 2004). WRN is also considered a caretaker-type tumor suppressor. As a gate-keeper, the p53 tumor suppressor promotes apoptosis that in principle would suppress carcinogenesis; however, such a pathway of cell death is attenuated in WS cells (Spillare et al., 1999). Epigenetic inactivation of WRN, due to CpG island promoter hypermethylation, has also been detected in some cancer cells (Agrelo et al., 2006). Although premature aging syndromes are phenocopies of normal human aging, clinical and molecular evidence strongly suggest that WS is a relevant model system for studying human aging and the relationship between aging and cancer.

2. Biochemical characteristics of WRN

WRN contains a 3′–5′ exonuclease domain, acidic regions, a 3′–5′ helicase domain, a RecQ C-terminal (RQC) domain, a helicase and ribonuclease D C-terminal (HRDC) domain, and a nuclear localization signal (NLS) (Fig. 1). Electrophoretic mobility shift assays using WRN domain fragments suggest that the RQC and HRDC domains of WRN fold independently and bind DNA in a structure-specific manner (von Kobbe et al., 2003b). The WRN RQC domain prefers to bind DNA structures resembling replication intermediates (forked and Holliday junctions) (von Kobbe et al., 2003b). The RQC domain also plays a role in WRN protein–protein interactions with FEN-1, BLM, TRF2, and PARP-1 (Brosh et al., 2001; von Kobbe et al., 2002, 2003a; Opresko et al., 2002). These DNA- and protein-binding features are consistent with recent structural studies of a WRN aa 949–1079 fragment that contains the WRN RQC domain (Hu et al., 2005), and a WRN aa 1142–1242 fragment that contains the HRDC domain (Kitano et al., 2006). Specifically, the RQC domain includes a ~20 aa winged helix subdomain that binds to DNA and protein (Hu et al., 2005). Amino acid substitution of Lys-1016 in the winged helix domain decreases WRN binding to fork or bubble DNA substrates and markedly reduces WRN helicase activity on fork, D-loop, and Holliday junction substrates (Lee et al., 2005). Additional biochemical studies are needed to verify the structural data that the winged helix domain also binds to proteins. Structural and biochemical studies were also recently performed using a human WRN aa 38–236 fragment or a mouse WRN aa 31–238 fragment that contains the exonuclease domain. The results of these studies suggest that the exonuclease domain of WRN belongs to the DnaQ family, which shares a conserved replicative proofreading 3′–5′-exonuclease (Perry et al., 2006; Choi et al., 2007). The results also suggest that WRN exonuclease activity is activated upon substrate DNA binding in a Zn^{2+}-dependent manner (Choi et al., 2007). This is consistent with results from biochemical assays demonstrating that Zn^{2+} and Mn^{2+} stimulate WRN exonuclease activity (Choudhary et al., 2004). To date, the structures of the helicase domain of WRN and full-length WRN have not yet been solved.

Members of the RecQ family share a conserved 3′–5′ helicase domain. Helicases separate complementary strands of nucleic acids in a reaction coupled to NTP hydrolysis. WRN is a DNA structure-specific helicase. The non-B form DNA G-quadruplexes and triple helix are highly preferred substrates for WRN helicase. The next most preferred substrates for WRN are DNA molecules that resemble recombination intermediates (D-loops, Holliday junctions, and three-way junctions), followed by structures associated with DNA replication (bubbles, forks, and flaps) and 3′-single-stranded DNA tailed dsDNA (Opresko et al., 2004a). One of the critical steps of the early response to DNA double strand breaks (DSBs) involves 5′-recession of blunt DNA ends to generate 3′-protruding ssDNA tails. Telomeres also have 3′-ssDNA tails that are thought to be folded and protected in the form of D-loops. It is notable that 3′-ssDNA tailed duplex is a good substrate for WRN helicase, but 5′-ssDNA tailed or blunt duplex molecules are not (Mohaghegh et al., 2001), suggesting that WRN may play an important role in telomere maintenance and DSB repair. This is consistent with the observation that the helicase activity of WRN is required for lagging strand DNA synthesis at telomeres (Crabbe et al., 2004).

Biochemical analyses indicate that the WRN helicase activity on forked duplex DNA is activated by telomeric binding proteins TRF2 and POT1 and by the MRN complex (Opresko et al., 2002; Cheng et al., 2004). It has also

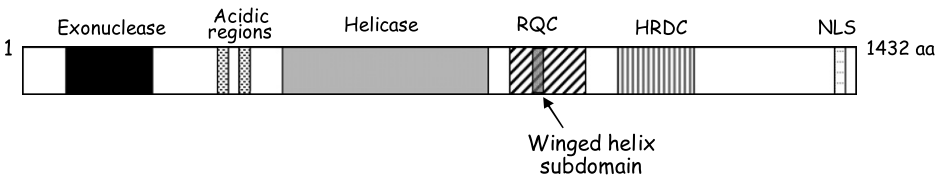

Fig. 1. Schematic representation of the domains of WRN. RQC, RecQ conserved C-terminal domain; HRDC, helicase and RNase D C-terminal domain; NLS, nuclear localization signal; aa, amino acids.