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Review

Roles of Werner syndrome protein in protection of genome integrity

Marie L. Rossi, Avik K. Ghosh, Vilhelm A. Bohr*

Laboratory of Molecular Gerontology, National Institute on Aging, Baltimore, MD 21224, United States

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ABSTRACT

Werner syndrome protein (WRN) is one of a family of five human RecQ helicases implicated in the maintenance of genome stability. The conserved RecQ family also includes RecQ1, Bloom syndrome protein (BLM), RecQ4, and RecQ5 in humans, as well as Sgs1 in *Saccharomyces cerevisiae*, Rqh1 in *Schizosaccharomyces pombe*, and homologs in *Caenorhabditis elegans*, *Xenopus laevis*, and *Drosophila melanogaster*. Defects in three of the RecQ helicases, RecQ4, BLM, and WRN, cause human pathologies linked with cancer predisposition and premature aging. Mutations in the *WRN* gene are the causative factor of Werner syndrome (WS). WRN is one of the best characterized of the RecQ helicases and is known to have roles in DNA replication and repair, transcription, and telomere maintenance. Studies both *in vitro* and *in vivo* indicate that the roles of WRN in a variety of DNA processes are mediated by post-translational modifications, as well as several important protein-protein interactions. In this work, we will summarize some of the early studies on the cellular roles of WRN and highlight the recent findings that shed some light on the link between the protein with its cellular functions and the disease pathology.

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Contents

Abbreviations: γ-H2AX, phosphorylated histone H2AX; 4-NQO, 4-nitroquinoline 1-oxide; 5-OHdC, 5-hydroxycytidine; 5-OHU, 5-hydroxyuracil; 8-oxodA, 8-oxoadenine; 8-oxodG, 8-oxoguanine; ALT, alternative lengthening of telomeres; APE1, apurinic/apyrimidinic endonuclease 1; ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3 related; BER, base excision repair; BLM, Bloom syndrome protein; CeWRN-1, *Caenhorhabditis elegans* WRN; CPT, camptothecin; D-loop, displacement loop; DmWRN, *Drosophila melanogaster* Werner syndrome protein; DSB, double strand break; DSBR, double strand break repair; ETO, etoposide; fapy, formamidopyrimidine; FEN1, flap endonuclease 1; FFA-1, focus forming activity 1; G4, G-quadruplex; HR, homolous recombination; HU, hydroxyurea; IR, ionizing radiation; LMNA, lamin A/C; MMR, mismatch repair; MMS, methylmethane sulfonate; NER, nucleotide excision repair; NHEJ, non-homologous end joining; PARP-1, poly (ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; Pol β, DNA Polymerase β; Pol δ, DNA Polymerase δ; PUVA, psoralen plus UVA; RNA pol I, RNA polymerase I; RNA pol II, RNA polymerase II; ROS, reactive oxygen species; RPA, replication protein A; Rrna, ribosomal RNA; SNPs, single nucleotide polymorphisms; SSB, single strand break; TPT, topotecan; WRN, Werner syndrome protein; WS, Werner syndrome; X4L4, XRCC4/Ligase IV.

* Corresponding author at: NIH Biomedical Research Center, 251 Bayview Boulevard, Baltimore, MD 21224, United States.
E-mail address: BohrV@grc.nia.nih.gov (V.A. Bohr).

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1. Overview

Werner syndrome protein (WRN) is one of a family of five human RecQ helicases implicated in the maintenance of genome stability. The conserved RecQ family also includes RecQ1, Bloom syndrome protein (BLM), RecQ4, and RecQ5 in humans (see additional reviews in this issue), as well as Sgs1 in *Saccharomyces cerevisiae*, Rqh1 in *Schizosaccharomyces pombe*, and homologs in *Caenorhabditis elegans*, *Xenopus laevis*, and *Drosophila melanogaster* [1]. Defects in three of the RecQ helicases, RecQ4, BLM, and WRN, cause human pathologies linked with cancer predisposition and premature aging [1–5]. Mutations in the *WRN* gene are the causative factor of Werner syndrome (WS). WRN is one of the best characterized of the RecQ helicases and is known to have roles in DNA replication and repair, transcription, and telomere maintenance [1–6]. Studies both *in vitro* and *in vivo* indicate that the roles of WRN in a variety of DNA processes are mediated by post-translational modifications, as well as several important protein–protein interactions [1,2,7]. Many of these functions of WRN in genome maintenance, as well as the clinical characteristics of WS, have been recently reviewed [8–12]. In this work, we will summarize some of the early studies on the cellular roles of WRN and highlight the recent findings that shed some light on the link between the protein with its cellular functions and the disease pathology.

2. Molecular genetics of Werner syndrome

WS is a rare autosomal recessive progeroid disorder characterized by the development of cataracts, changing of skin conditions, bird-like facies, atypical short stature, and premature graying or thinning of the hair [9]. Patients also often develop hypogonadism, osteoporosis, diabetes mellitus, artherosclerosis [13], and cancers, particularly sarcomas [14]. Onset of symptoms usually occurs in the third decade of life, and health subsequently declines with median age at death between 47 and 54 years [13]. Because WS presents with early-onset of conditions commonly seen in the aged, it is a good model system for the study of mechanisms of normal aging [15,16].

2.1. WRN biochemistry

The causative factor of the majority of WS is mutation in the *WRN* gene, which codes for a member of the highly conserved RecQ family of helicases. While several different mutations within the gene are seen in WS, most result in production of truncated WRN protein [13]. Human WRN possesses 3′-5′ATP-dependent helicase, 3′-5′ exonuclease, and single-stranded DNA annealing activities [17–23]. Early gel filtration chromatography studies indicated that purified full length WRN exists as a trimer [24], and a fragment containing only the exonuclease domain equilibrates between a trimeric and hexameric form [25]. On the other hand, current electron microscopy data indicates that WRN is likely found as a dimer in solution, yet behaves as a tetramer in complex with DNA [26]. Still, while unwinding DNA, WRN acts as a monomer [27]. Together these results suggest that WRN's oligomeric state may be dependent on whether or not it is catalytically active and how it is interacting with DNA.

Orthologs of human WRN have been identified recently and characterized in other eukaryotes, including *Caenorhabditis elegans* (CeWRN-1) [28,29], *Drosophila melanogaster* (DmWRN) [30–32], and *Xenopus laevis* (focus forming activity 1 (FFA-1)) [33]. *Xenopus* FFA-1 is active as a helicase, while the exonuclease has not yet been investigated [33]. Similarly, the CeWRN-1 is active as a 3′-5′ATP-dependent helicase, though it lacks the 3′-5′ exonuclease [28]. In contrast, *Drosophila* DmWRN is active as a 3′-5′ exonuclease [30], but the WRN helicase homolog has yet to be identified [31,32]. Nevertheless, cellular studies indicate that depletion or mutation of WRN increases sensitivity to DNA damage and results in increased genome instability [29–32]. While the enzymatic activities of the WRN orthologs in *Xenopus*, *C. elegans*, and *Drosophila* may not be entirely conserved with the human WRN, each is clearly important for genome maintenance.

WRN is active in resolving a variety of DNA substrates, including forks, flaps, displacement loops (D-loops), bubbles, Holliday junctions, and G-quadruplexes (G4), all of which represent intermediates in DNA replication and repair processes [34,35]. The roles of WRN in these pathways will be described later in detail. Importantly, the enzymatic activity of WRN on DNA substrates can be modulated by post-translational modifications [7]. The helicase and exonuclease activities of WRN are inhibited by oxidation [36]. WRN catalytic activity is also inhibited by both DNA-PK serine/threonine phosphorylation [37,38] and c-Abl tyrosine phosphorylation [39]. Conversely, p300 acetylation stimulates WRN helicase and exonuclease [40]. Additionally, WRN is subject to sumoylation, although the affect of this on its activities has yet to be determined [41]. Presumably, post-translational modification of WRN regulates its roles in multiple DNA transactions.

2.2. Epigenetic regulation and cancer

Recent studies have also shown an epigenetic component of WRN regulation [42,43]. Epigenetic regulation, specifically hypermethylation of tumor suppressor genes, is tightly linked with cancer [44]. In primary tumors, hypermethylation of the *WRN* promoter is common and correlates with lower levels of WRN protein expression. This hypermethylation inactivates the gene and results in increased chromosomal instability [42]. Inactivation of WRN in cancer cells increases susceptibility to the cytotoxic effects of topoisomerase inhibitors commonly used as chemotherapeutic agents [42,45]. Moreover, knockdown of WRN protein expression induces cell death and growth arrest in several human cancer cell lines, consistent with a tumor-suppressor like role of WRN. Conversely, following WRN depletion, cells that persist in growth have detectable levels of WRN protein indicating that WRN may also promote tumor proliferation [43]. It is likely that the roles of WRN in preserving genome stability and facilitating DNA repair must be balanced to allow for normal cell growth while protecting against cancer formation [43]. Together, these results suggest that WRN is a potential target for anti-tumor therapies [42,43,45].

In addition to the general association of WRN protein expression with cancer, recent analyses of *WRN* single nucleotide polymorphisms (SNPs) show a connection with cancer susceptibility [46,47]. An increased risk of breast cancer is seen among Chinese patients with the *WRN* Leu1074Phe SNP [46] and among German