[Frontiers in Bioscience 11, 2657-2671, September 1, 2006]

Werner syndrome: molecular insights into the relationships between defective DNA metabolism, genomic instability, cancer and aging

David K. Orren

Graduate Center for Toxicology, University of Kentucky College of Medicine, Lexington, Kentucky

TABLE OF CONTENTS

1. ABSTRACT

Werner syndrome is a segmental progeroid disease characterized by increased cancer and acceleration of specific age-related phenotypes, due to loss of a protein known as WRN. Extensive research over the last decade has revealed much about WRN biochemistry and the etiology of Werner syndrome. WRN possesses multiple DNA-dependent enzymatic activities (ATPase, helicase, exonuclease, and strand annealing) and interacts with factors having established roles in DNA metabolic pathways. Although the exact functions of WRN remain unclear, accumulating evidence points to roles in proper resolution of replication blockage and in telomere maintenance. If WRN function is lost (as exemplified in cells from Werner patients), problems with replication and DNA damage processing arise, probably resulting in an increased number or persistence of strand breaks. In turn, these events lead to chromosomal and telomeric abnormalities or activate checkpoints that bring about early senescence or increased apoptosis. Thus, elevated cancer incidence associated with Werner syndrome is due to increased chromosomal changes, while the accelerated aging characteristics probably stem from telomere dysfunction leading to accumulation of non-functional senescent cells or excessive apoptotic cell death over time. More research is needed to determine whether these specific DNA-dependent mechanisms contribute to development of aging characteristics in normal individuals.

2. INTRODUCTION

A large body of research has clearly shown that genomic instability plays a key role in tumor initiation and progression. Human hereditary diseases that show increased genomic instability and elevated cancer incidence have provided valuable insight into the cellular processes that suppress genetic change and therefore, carcinogenesis. In fact, much of our current knowledge about DNA repair and checkpoint pathways in humans stems from research into the molecular bases of these genomic instability syndromes. Even with these protective mechanisms intact, genetic change in cells still occurs over time and thus the probability of cancer increases with age. This raises the critical question of whether genetic change might be involved in the development of aging phenotypes other than cancer. In support of this hypothesis, some cancer-prone hereditary diseases also show accelerated development or increased frequency of other characteristics or pathologies normally associated with aging. Of these diseases, Werner syndrome (WS), first identified by Otto Werner in 1904 (1), has gained increased attention recently as an excellent model system for examining the potential relationship between genomic instability and aging.

WS or “adult progeria” is an autosomal recessive segmental progeroid disease, showing accelerated development of some but not all features of normal aging (2,3). In contrast to other progeroid syndromes, individuals

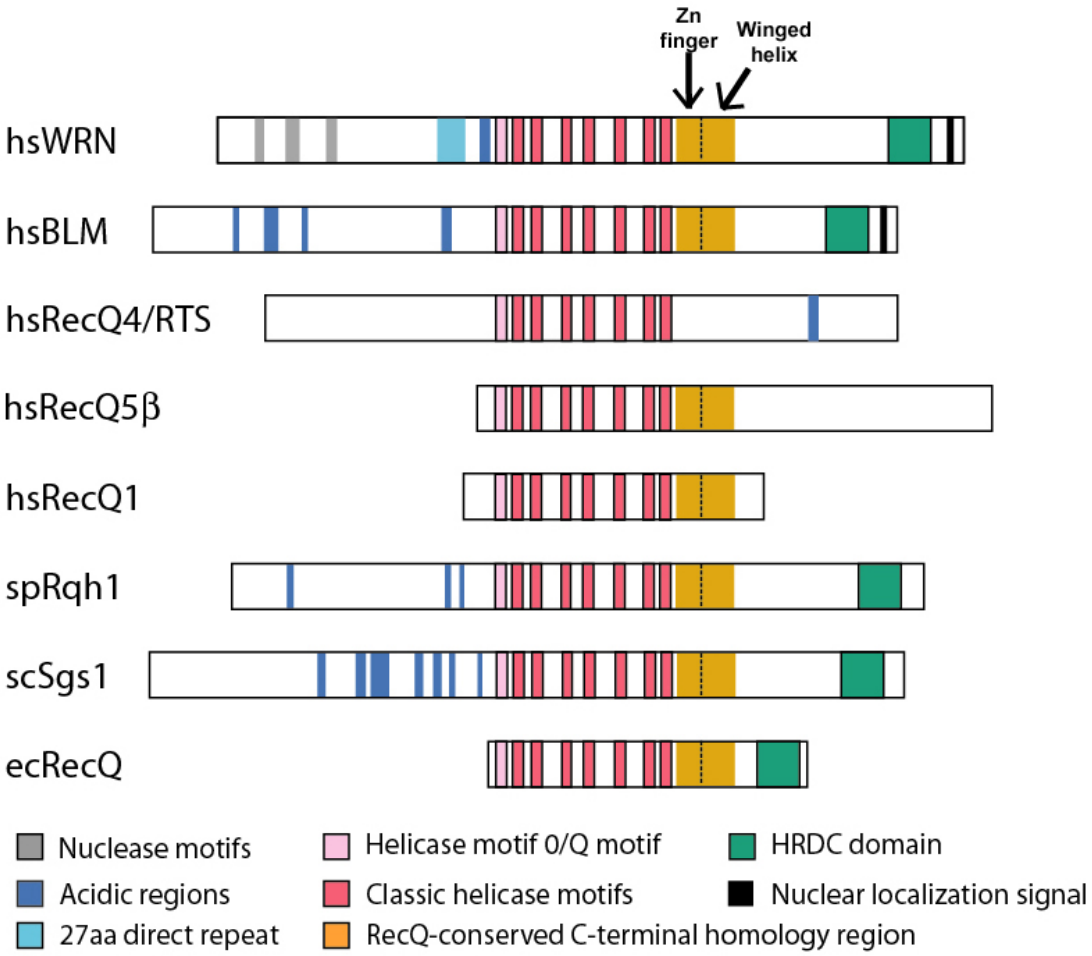

Figure 1. WRN and other key RecQ family members. The human RecQ family members (hsWRN, hsBLM, hsRecQ4, hsRecQ5β and hsRecQ1) along with spRqh1, scSgs1, and ecRecQ, the lone RecQ homologs from *S. pombe, S. cerevisiae, and E. coli*, respectively, are aligned with respect to their conserved ATPase/helicase motifs (*in red*). Other domains of interest are depicted and identified by the guide at bottom. The RecQ-conserved C-terminal homology region or RQC domain (*in orange*) is bisected (*dotted line*) into Zn finger and winged helix subdomains as indicated above the WRN protein map.

with WS are asymptomatic in early childhood and often go undiagnosed until beyond twenty years of age. This relatively normal developmental period suggests that WS might be a better model for aging research than other progeroid syndromes that have earlier onset phenotypes arising from severe developmental defects. During their teenage years, individuals with WS fail to undergo the normal adolescent growth spurt resulting in generally short stature. With time, they also begin to show accelerated development or increased frequency of many age-related features including cataracts, atherosclerosis, osteoporosis, hypogonadism, diabetes mellitus type II, hypertension, graying and loss of hair, and wrinkling of the skin. As for most diseases showing increased genomic instability, an elevated incidence of cancer is associated with WS. However, there is a marked increase in tumors derived from tissues of mesenchymal origin (soft tissue sarcomas and osteosarcomas) in WS, perhaps indicating that these cell types are particularly vulnerable to the molecular defect(s) underlying the syndrome. Unlike normal aging, no obvious advancement of neurodegeneration is associated with WS, an outcome possibly related to the early differentiation and post-mitotic status of neural tissue. Individuals with WS generally die before age 50 either from cancer or heart disease. Although WS does not display accelerated development of the total spectrum of normal aging characteristics, the specific phenotypes observed in WS provide a good starting point for examining a potential role for genomic instability in normal aging.

3. GENETIC BASIS OF WS

Another reason that WS has been touted as an excellent model for aging research is its genetic simplicity. Amazingly, all of the WS phenotypes mentioned above result from defects in both alleles of a single gene, known as *WRN*, located at chromosome position 8p12 (4,5). At its very C-terminus, WRN contains a nuclear localization sequence (Figure 1). Intriguingly, all of the *WRN*

Table 1. Proteins and complexes that interact with WRN in human cells

Protein/Complex	Established Functions	References
RPA	Replication & Repair	30,36,37,77
PCNA	Replication & Repair	75,95
DNA polymerase δ	Replication & Repair	96
FEN-1	Replication & Repair	97
Topoisomerase I	Replication	95
WRNIP1	Replication	146
RAD51	Recombinational Repair	77
RAD52	Recombinational Repair	40
BLM	Recombinational Repair	61
TRF2	Telomere Structure & Function	39,59,141,142
POT1	Telomere Structure & Function	41
Ku heterodimer	DNA Break Binding (NHEJ, Telomere maintenance)	54,55,58
PARP-1	DNA Break Binding & ADP-Ribosylation	103-105
DNA-PKcs	NHEJ Kinase	65,67,102
ATR	DNA-dependent Checkpoints	68
MRN complex	DNA-dependent Checkpoints	78
p53	DNA-dependent Checkpoints & Transcription	38,60
gamma-H2AX	DNA-dependent Checkpoints & Chromatin Remodeling	79
NDH II	RNA processing, Transcription	56
PSO4	mRNA Splicing, Crosslink Repair	111

mutations identified in individuals with WS truncate the gene product prior to this nuclear localization signal (6,7). This has led to speculation that the observed aging phenotypes result from the total absence of WRN function due to the inability of these truncated proteins to enter the nucleus. Consistent with the idea of a null phenotype, the truncated versions of WRN conferred by patient mutations are not generally detectable in cells, probably due to their improper conformation and rapid degradation (8,9). Thus, the confluence of premature aging phenotypes in WS patients with markedly different mutations is likely explained by a complete loss of WRN function.

Since a complete absence of WRN function leads to a profound acceleration of specific aging phenotypes, it is tempting to speculate that either altered WRN expression (for example, due to loss of one allele) or various combinations of WRN polymorphisms or missense mutations may influence the time of onset or frequency of specific aging features even in phenotypically normal individuals. Consistent with the former possibility, cells from WRN heterozygotes with one null allele have intermediate phenotypes regarding DNA damage sensitivity (10). In support of the latter, a specific polymorphism resulting in a Cys to Arg change at amino acid residue 1361 correlates to a higher rate of osteoporosis in a Japanese population while the Arg allele seems to be protective against myocardial infarction (11,12). However, this same polymorphism did not either alter WRN function *in vitro* or result in differences in coronary artery disease in a Caucasian population (13), suggesting possible effects of genetic background on WRN function.

4. THE *WRN* GENE PRODUCT

4.1. Primary sequence and structural information

The *WRN* gene product encodes a protein of 1432 amino acids with an approximate molecular weight of 162 kilodaltons and, based on sequence motifs in its central region, belongs to the RecQ family of helicases (5). RecQ family members have important roles in maintaining large-scale genomic stability, as loss of their individual functions results in increased chromosomal rearrangements that are probably caused by erroneous joining (misrejoining) of unrelated DNA double strand breaks. Prokaryotes and lower eukaryotes possess only a single RecQ helicase, while higher eukaryotes have multiple RecQ family members. Humans have five RecQ helicases (Figure 1), including the *BLM* and *RECQ4* gene products that are defective in the cancer-prone Bloom and Rothmund-Thomson syndromes, respectively (14,15). Furthermore, certain mutations in *RECQ4* cause RAPADILINO or Baller-Gerold syndrome, each phenotypically similar to Rothmund-Thomson but apparently with significantly lower cancer risk (16,17). Although the human RecQ family members are highly homologous within the sequence motifs that comprise their helicase domains, other regions outside of this domain are mostly dissimilar (Figure 1). However, there are two regions of weak homology (the RQC and HRDC domains) C-terminal to the helicase domain that are shared between WRN and BLM orthologs as well as some other RecQ family members. A recent crystal structure of *Escherichia coli* RecQ (18) has demonstrated that the RQC domain is divided into two structural entities, a Zn finger and a winged helix domain. Studies with isolated RQC and HRDC domains from WRN and other RecQ members indicate that each domain folds into a distinct structural entity with DNA binding affinity (19-22). WRN is the only human RecQ member to have three sequence blocks in its N-terminal region that are typical of nucleases (23), suggesting that loss of not only its helicase but also its exonuclease activity (see below) contributes to the unique WS phenotype. It is notable that WRN orthologs containing both nuclease and helicase domains have been identified in mouse (mWrn) and *Xenopus laevis* (FFA-1/*xl*WRN) but not in prokaryotes, lower eukaryotes, or *Drosophila melanogaster* (24,25). However, there are indications that in plants and lower eukaryotes, WRN-like exonuclease and helicase functions may reside in separate genes (26).

4.2. Association of WRN with Other DNA Metabolic Factors

WRN has been shown to associate or interact directly with many factors that participate in DNA metabolism or are involved in the cellular response to DNA damage. A list of factors that interact with WRN is presented in Table 1. A complete description of each of these interactions and their potential relevance to WRN function is beyond the scope of this review. However, efforts have been made in the following sections to refer to specific interactions that may influence either WRN activities *in vitro* or its potential DNA metabolic roles *in vivo*.

4.3. ATPase and Helicase Activities

As predicted from its strong homology to RecQ helicases and weaker homology to a very large family of ATPase and nucleic acid unwinding enzymes, the central region of WRN confers ATPase activity that provides the

energy for unwinding nucleic acids. Not surprisingly, deletions that remove or partially truncate the helicase domain and substitution mutations at conserved residues (K577M and R834C) in the ATPase/helicase motifs disable ATPase and unwinding activities (27,28). The ATPase activity of WRN is essentially undetectable unless DNA (or RNA) is present, with single-stranded DNA optimally stimulating this activity (29). WRN alone unwinds short regions of duplex DNA with a 3' to 5' directionality in a reaction mediated by ATP hydrolysis; dATP, CTP, or dCTP can substitute (at least partially) for ATP in driving WRN-mediated unwinding (30). The helicase activity of WRN appears especially robust on unusual DNA structures including triplexes, 2-, 3- and 4-stranded fork structures and 3-stranded displacement (D-)loops (31-34). In addition, this activity mediates branch migration of Holliday junctions and disruption of G-quartet structures that might occur in guanine-rich regions including telomeres and ribosomal DNA arrays (31,35,36). It is notable that the DNA substrate specificity for WRN helicase activity is remarkably similar to that of the human BLM protein. WRN alone is relatively weak helicase, being able to unwind duplexes longer than 40 bp only with extreme difficulty; this may partially be due to the recently identified annealing activity (see below) also present in WRN that tends to counteract simple unwinding. However, a direct interaction with replication protein A (RPA), the human single-stranded binding factor, permits WRN to unwind much longer duplexes (up to several hundred bp) in a manner not duplicated by heterologous single-stranded binding proteins (30,37). Other interacting proteins (such as p53, RAD52, TRF2, and POT1) have been shown to regulate WRN unwinding activity either positively or negatively (38-41).

4.4. Annealing Activity

WRN also facilitates the pairing of complementary DNA strands (42). This appears to be a property shared between multiple RecQ family members, including BLM, RecQ1, RecQ5β, and *Drosophila melanogaster* RecQ5b (42-45). Although it has been suggested that conserved C-terminal regions (RQC and HRDC) of these proteins are necessary for annealing, the structural basis for this activity remains unclear. WRN pairs complementary single-stranded oligomers (at greater than 250-fold the spontaneous rate at sub-nM DNA concentrations) in a manner that directly opposes unwinding, suggesting that the actual WRN unwinding strength may have been underestimated (42,46). WRN also mediates pairing of third strands to complementary single-stranded regions in fork and bubble substrates; on forked structures, coordination between pairing and unwinding promotes strand exchange (42). It seems likely that such coordination between pairing and unwinding activities of WRN may be responsible for its strong branch migration activity (36) and possibly other recombination-related properties.

4.5. Exonuclease Activity

The nuclease domain at the N-terminus of WRN confers a 3' to 5' exonuclease activity to the full length protein (47,48). Mutations at conserved residues in this domain (D82A and E84A) specifically eliminate this activity (47). On partial duplex structures, the exonuclease activity of WRN appears most robust on recessed and mismatched 3' ends (49). WRN preferentially digests 3' ends or nicks within complex structures containing bubbles, D-loops, stem-loops, and Holliday junctions that are also excellent substrates for its helicase function (34,50,51). The isolated N-terminal domain of WRN retains 3' to 5' exonuclease activity (51,52) and may have endonuclease activity under certain conditions (53), indicating that it folds into a functional unit that is structurally separate from the remainder of the protein. The exonuclease activity of WRN can be stimulated by its interaction with Ku heterodimer or NDH II (54-56); Ku allows WRN to degrade DNA containing minor base lesions that normally block the exonuclease activity of WRN alone (57,58). WRN exonuclease activity is specifically enhanced on telomeric substrates by an interaction with TRF2 (59). In contrast, WRN exonuclease activity is inhibited by p53 or BLM (60,61).

4.6. DNA Binding Activity

As might be expected from an enzyme with helicase and exonuclease activity, WRN has DNA binding capability. In the absence of interacting proteins, the binding affinity of WRN appears to be dependent upon DNA structure with no apparent nucleotide sequence preference. WRN binds better to single-stranded than double-stranded DNA, and without preferential affinity to DNA containing oxidative damage, UV-induced photoproducts, or 4-nitroquinoline-1-oxide (4NQO) adducts (29). Recently, WRN was shown to bind to single-stranded DNA in a manner influenced by substrate length (62). Electrophoretic mobility shift assays show that WRN binds most stably to unusual DNA structures, with highest affinity for substrates containing bubbles, unpaired loops, stem-loops, and D-loops (34,50). Experiments using truncated versions of WRN show that four distinct regions have DNA binding properties, including the isolated helicase, RQC, and HRDC domains; the exonuclease domain also binds DNA, but more weakly (20,62). Thus, varying levels of exonuclease and helicase activities on different DNA structures likely stem from binding preferences conferred by the helicase domain and C-terminal regions of WRN. This is also consistent with structural information obtained on specific domains of WRN and other RecQ family members (18,19,21,63,64). In general, the existing evidence suggests that the 3-dimensional conformation of WRN is organized to bind multiple sites on 3- or 4-stranded DNA structures, most likely replication or recombination intermediates.

4.7. Modification and Subcellular Localization of WRN

As for countless other proteins, the cellular function of WRN appears to be regulated by post-translational modifications. Although some WRN phosphorylation may be detected in undamaged cells, DNA replication inhibitors and certain DNA damaging treatments trigger acetylation and increased phosphorylation of WRN (65-69). The acetylation and phosphorylation status of WRN appears to regulate its trafficking between subnuclear compartments (see below).

In addition, phosphorylation of WRN has been shown to modulate its exonuclease and helicase functions (65,67). WRN phosphorylation after replication arrest is mediated by primarily an ATR-dependent pathway, while phosphorylation in response to DNA damage may be mediated (possibly in a redundant manner) by ATR-, ATM- or DNA-PK-dependent pathways (67,68). These phosphotidylinositol-3-kinase family members phosphorylate serine and threonine residues specifically at SQ and TQ sequences in their target proteins (70). WRN contains eight such sequences, six of which are located C-terminal to the conserved helicase domain. Notably, a synthetic peptide derived from WRN including serine 1141 was shown to be preferentially phosphorylated by ATM, ATR, and DNA-PK (71). In addition, tyrosine phosphorylation of WRN has been observed following treatment with mitomycin C, but the responsible kinase(s) are unknown (69). Ectopically expressed WRN is modified by ubiquitin-like SUMO-1 molecules within cells (72), although the putative function of this modification remains unclear.

Immunofluorescence studies indicate that WRN is localized within the nucleus, consistent with its putative roles in DNA metabolism. However, its subnuclear distribution varies depending upon cellular circumstances. In unperturbed proliferating cells, WRN is diffusely distributed throughout the nucleus with, in some circumstances, a more intense association with the nucleolus that may be regulated by its phosphorylation status (9,69,73). In a subset of proliferating cells, discrete nuclear foci containing WRN are sometimes observed; such foci are found in cells in S phase and correspond to DNA replication "factories" (74,75). In quiescent cells, WRN may become dissociated from the nucleolus (73). Curiously, mouse WRN shows only nucleoplasmic (and not nucleolar) localization, perhaps due to its lack of a nucleolar targeting sequence that is present in human WRN (9,76).

Treatment of cells with certain DNA damaging agents or replication inhibitors also alters the subnuclear localization of WRN. WRN relocalization can be triggered by treatment of cells with ionizing radiation, bleomycin, hydrogen peroxide, 4NQO, mitomycin C, methylmethane sulfonate, camptothecin, etoposide, hydroxyurea (HU), and UV irradiation (to a much smaller degree) (58,69,73,77). In these instances, a significant amount of WRN migrates into discrete nuclear foci that correspond to sites of ongoing or arrested DNA replication (74,75,77). Other DNA metabolic factors found in these foci include PCNA, RPA, RAD51, RAD52, ATR, the MRN complex and Ku (36,40,58,68,74,77-79). This redistribution to discrete foci following DNA damage appears to correlate with lysine acetylation of WRN (66,69) and is reversible, with WRN returning to its predominant nucleolar localization after varying intervals depending upon the agent used (69).

5. WS CELLULAR PHENOTYPES AND POTENTIAL ROLES FOR WRN IN DNA METABOLISM

5.1. Genomic Instability and DNA Damage Hypersensitivity

Cells from WS patients have an elevated frequency of spontaneous chromosomal aberrations characterized by deletions, insertions, and translocations (80-82), a phenotype undoubtedly responsible for at least the increased cancer disposition associated with the disease. These types of mutations are probably due to misrejoining of unrelated double-strand breaks by non-homologous end joining (NHEJ) pathways. This particular genomic instability phenotype probably results from an increased number or longer persistence of DNA strand breaks. Consistent with this notion, the numbers of DNA breaks that occur spontaneously or persist following treatment with specific DNA damaging agents (4NQO, topoisomerase II inhibitor VP16, and topoisomerase I inhibitor camptothecin) are higher in WS cells than in normal cells (81,83,84).

Since cells from certain hereditary syndromes with genomic instability and increased cancer frequency were found to have defined defects in DNA damage processing, WS cells have also been extensively investigated for cell survival following various DNA damaging treatments. Among the many agents tested, cells with WRN deficiencies are hypersensitive to the effects of 4NQO, camptothecin, interstrand crosslinking agents including mitomycin C, cisplatin, and mephalan, and Me-Lex, an alkylating agent that predominantly generates 3-methyladenine lesions (10,24,84-88). Intriguingly, survival following cisplatin treatment appears to be normal if WRN proteins having only helicase or only exonuclease activity are expressed (89). Although various reports are somewhat contradictory, there may also be a modest sensitivity of WS cells to ionizing radiation (65). There is no apparent hypersensitivity of WS cells to UV irradiation (90), the agent of choice when examining potential deficiencies in nucleotide excision repair (NER). In assessing these findings, it must be kept in mind that hypersensitivity as measured by cell survival can involve much more than just the efficiency of DNA adduct removal. In fact, the structurally diverse lesions generated by these agents are subject to direct removal by disparate repair pathways (NER, base excision repair, double-strand break repair), suggesting that WRN is not essential for any one of these specific repair pathways. Instead, these results support the idea that WRN is involved in an alternate (backup) pathway that either removes lesions or allows the cell to replicate and survive in the presence of damage, perhaps a mechanism that takes effect when persistent lesions with dissimilar structural properties block the progression of replication forks. In this regard, the established hypersensitivity of WS cells to HU is particularly illustrative (84,91), since HU inhibits replication fork progression by depleting nucleotide pools and not by generating polymerase-blocking adducts. Notably, the observed hypersensitivities of WS cells following DNA damaging or replication blocking treatments appear to result specifically from apoptotic cell death of S or G_2 phase cells (84).

5.2. DNA Replication, Repair, and Recombination Abnormalities

Even in the absence of exogenous DNA damage, cells derived from WS patients have longer cell division cycles than their normal counterparts. This longer division

cycle is apparently due to a specific extension of S phase and a slower overall rate of DNA replication (90,92,93). Examination of DNA replication properties in WS cells has revealed an increase in replicon size (94) and asymmetry in the progression of bidirectional replication forks (74). Consistent with a possible role in DNA replication, WRN has been isolated as part of a DNA replication complex and has been shown to interact with a number of factors directly involved in DNA replication including DNA polymerase δ, PCNA, RPA, and FEN-1 (37,75,95-97). Furthermore, WRN can be found associated with nuclear foci that correspond to sites of deoxynucleotide incorporation (74,75). Although not required for DNA replication in nuclei from reconstituted *Xenopus* egg extracts, the WRN ortholog FFA-1 also is involved in formation of discrete replication foci (25,98). WRN expression is upregulated during S and G_2 phases in highly proliferative, transformed cell lines, suggesting that its function may be tied to DNA replication (99). Taken together, this data suggests that WRN may play a non-essential but important role in optimizing DNA replication.

The nature of genomic instability observed in WRN-deficient cells suggests improper processing of DNA breaks that are normally repaired by NHEJ or homologous recombination (HR). In line with this notion, deletions at the rejoining point in plasmid-based assays are much more extensive in WS cells than normal cells even though rejoining efficiency is not compromised (100,101). A role for WRN in processing of DNA breaks may be facilitated by its physical and functional interactions with two factors, PARP1 and the Ku heterodimer, that bind directly to DNA breaks, the latter either alone or in conjunction with the DNA-dependent protein kinase (DNA-PK) complex that contains Ku and the DNA-PK catalytic subunit (54,55,58,65,67,102-105). There is also considerable evidence for the participation of WRN in recombinational repair, including a large body of research on RecQ, Sgs1 and Rqh1, the lone RecQ family members in *Escherichia coli*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively (reviewed in 106-109). Direct examination of WRN-deficient cells revealed a defective mitotic recombination phenotype (110). A more in-depth molecular and genetic analysis suggested that WRN might be involved in Holliday junction resolution during HR, as introduction of a heterologous resolvase complemented an intrachromosomal recombination deficiency associated with WS cells (91). It is of note that both the helicase and exonuclease activities of WRN are required to achieve normal levels of recombination using this intrachromosomal recombination assay (89). In addition to the aforementioned hypersensitivity of WS cells to DNA crosslinking agents, recent molecular studies (111) suggest a functional role for WRN in interstrand crosslink repair pathways that may also have a recombinational component. Research with the *Xenopus* system suggests that WRN might participate in single-strand annealing, a pathway that may be important for repairing DNA breaks specifically within repetitive regions of DNA (112). Although the genomic instability phenotype of WS cells implies a problem in suppressing the aberrant rejoining of double-strand breaks, an essential role for WRN in strand break repair seems unlikely, due to the relative insensitivity of WS cells (compared to cells deficient in HR factors, PARP1 or subunits of DNA-PK complex) to agents that induce these highly deleterious lesions in DNA.

The sometimes discordant observations from WRN-deficient cells might be explained by a defective response to blockage of replication fork movement. Replication blockage may be a relatively common event, caused by persistent endogenous or exogenous damage or secondary structures in the DNA template. It has been recognized within the last decade that the completion of replication often necessitates recombinational functions, ostensibly to deal with blocked replication forks and restart replication (113-117). As for some other RecQ-deficient conditions, the phenotypes of WS cells are consistent with a possible role for WRN in pathways that help overcome replication blockage and restart replication to both allow cell survival and minimize genomic instability. In the absence of WRN function, replication blockage may lead to increased DNA strand breaks that are rejoined in error-prone manners, possibly explaining the genomic instability phenotypes of WS cells. Moreover, the lowered survival of WS cells following certain DNA damaging treatments may be due to the inability to overcome replication blocking events leading to S and G_2 checkpoint activation and eventually resulting in apoptotic cell death.

5.3. Premature Cellular Senescence

Cellular or replicative senescence due to permanent exit from the cell cycle was first recognized for primary fibroblasts in culture by Hayflick (118) and has since been touted as a potential mechanism in the development of aging characteristics (119-121). This connection between cellular senescence and aging has received considerable support due to the abnormal growth kinetics of cells derived from individuals with WS. WS primary skin fibroblasts undergo premature cellular senescence after only about 15-25 population doublings (PDLs), compared to, on average, 50-80 PDLs for normal primary fibroblasts (122,123). Although senescence can be reached via different molecular pathways, a prominent route is through shortening and deprotection of telomeres, the highly repeated sequences at chromosome ends. The senescence of WS fibroblasts is quite similar to that of normal fibroblasts undergoing telomere-mediated senescence, except that WS cells apparently retain *c-Fos* inducibility (124,125). Introduction of the telomere maintenance enzyme telomerase prevents the replicative senescence of WS primary fibroblasts (126-128). This result has been interpreted in two different ways: 1) telomerase promotes takeover of cultures by clonal division of remaining cells that have not activated a senescence checkpoint (129) or 2) WS cells have a telomere maintenance defect (see below) that is rescued by telomerase expression.

5.4. Abnormal Telomere Dynamics

There is accumulating evidence that WRN has a specific function in telomere maintenance, and that loss of this function is responsible for the premature onset of the specific aging phenotypes presented in WS. Most somatic

cells (including primary fibroblasts) do not express detectable levels of the telomere lengthening enzyme telomerase, and thus the telomeric ends of their chromosomes may be depleted over time by either gradual shortening due to the end replication problem or stochastic deletions. Changes in telomeric structure resulting from critically shortened and/or unprotected telomeres expose the chromosome ends as DNA double-strand breaks, triggering ATM- and p53-dependent checkpoint pathways and subsequently either senescence or apoptosis (130,131). Thus, it has been suggested that telomere shortening serves as a clock to limit the number of divisions that cells can undergo, a mechanism that is widely believed to suppress tumorigenesis. After this connection between telomere dysfunction and at least certain instances of cellular senescence was established (132), investigations of telomere dynamics in cells lacking WRN function began in earnest. Studies of telomeric restriction fragment length in genomic DNA isolated from WS primary fibroblasts showed a faster rate of telomere erosion than normal fibroblasts, but WS cells senesced with somewhat longer telomeres, on average, than normal cells (133). When similar assays were performed in immortalized cells, normal cells showed typical telomere erosion profiles, while WS cells showed telomere lengths that fluctuated dramatically over time (134). Importantly, introduction of telomerase into WS fibroblasts essentially resulted in their immortalization (126-128), providing a direct link between possible telomere dysfunction in these cells and their premature senescence phenotype.

This link has been further strengthened by recent studies on mice lacking both telomerase and mWrn. Generation of mWrn-deficient mice was somewhat disappointing in that no overt premature aging or cancer-prone phenotypes were observed (24,135,136). However, strains generally used for generation of transgenic and knockout mice have very long (20-50 kb) telomeres, and even a complete deficiency of telomerase activity causes no overt phenotype until six or more generations (137). In this light, mWrn function was hypothesized to be less important or even dispensable in mice with very long telomeres and intact telomerase. To examine whether mWrn function might be important when telomere length was limited, telomerase-deficient mice were crossed with mWrn-deficient mice. When late generation (generation 3-5) telomerase-deficient mice containing shortened telomeres were used, an additional mWrn-deficiency resulted in replicative senescence at the cellular level and appearance of premature aging phenotypes (cataracts, osteoporosis, alopecia, and elevated mesenchymal tumors) reminiscent of human WS (138,139). When cells taken from these doubly-deficient mice were passaged in culture, survivors that escape from senescence arose at an elevated frequency, and these survivors showed evidence of telomeric recombination including an upregulation of the alternative lengthening of telomeres (ALT) pathway (140). This suggests that mWrn might act in a pathway that directly or indirectly suppresses telomeric recombination.

Other evidence also supports a specific function for WRN in telomere metabolism. WRN co-localizes with the telomeric repeat binding factors TRF1 and TRF2 (141,142) and telomeric DNA in late S phase (142,143) and co-immunoprecipitates with TRF2 (39). Expression of a dominant-negative, helicase-deficient WRN mutant protein in normal cells results in stochastic loss of telomeric sequences and chromosome fusions (143,144). One of these reports (143) suggests that these telomeric deletions result from a problem in replication of the lagging (G-rich) strand of telomeres. Biochemical studies demonstrate a direct physical and functional interaction between WRN and TRF2 (59,142). This interaction serves to enhance the helicase and exonuclease activities of WRN particularly on DNA substrates with telomeric character. These data are consistent with a model by which TRF2 recruits WRN to its site of action on telomeric structures (59). Recently, a functional interaction was observed between WRN and POT1, another telomeric protein that specifically binds to the single-stranded (G-strand) overhang at the telomeric ends (41).

Although additional roles are possible, this evidence strongly suggests that WRN functions in telomere maintenance. Hypothetically, telomere dysfunction occurs earlier and more often when WRN is absent, triggering activation of checkpoint pathways that result in senescence or apoptosis at an increased frequency during cellular life span. Transfection of WRN-deficient cells with telomerase rescues this defect, presumably by adding telomeric sequences *de novo* to depleted telomeric ends and preventing the activation of checkpoint pathways. What role might WRN play at telomeres? It seems likely that, at the molecular level, loss of WRN results in perhaps occasional telomeric deletions that can shorten individual telomeric ends by varying degrees. This phenotype may be due to 1) loss of direct effect of WRN in suppressing intra- or intertelomeric recombination events, 2) loss of the ability to properly resolve telomeric recombination events, or 3) loss of an effect of WRN during replication of telomeric regions such as disruption of telomeric secondary structures (G-quartets or telomeric loops) that directly truncates telomeric regions or indirectly increases the frequency or necessity of telomeric recombination. With regard to the third mechanism, it is notable that recombination pathways are thought to be essential to overcoming or circumventing replication blocks and reforming a functional replication fork.

6. PERSPECTIVE

Similar to the genomic instability phenotypes of other RecQ-deficiencies, the WS cellular phenotypes suggest that WRN function is important for minimizing large-scale chromosomal aberrations. However, WS is certainly phenotypically unique, with a much higher prevalence of premature aging phenotypes but a lower cancer frequency when compared with Bloom syndrome. This would suggest that the DNA metabolic functions of WRN are distinct from or at least only partially redundant with other human RecQ helicases, even though the WRN and BLM proteins have many enzymatic similarities. At this time, the preponderance of evidence points to functions for WRN in proper resolution of telomere maintenance,

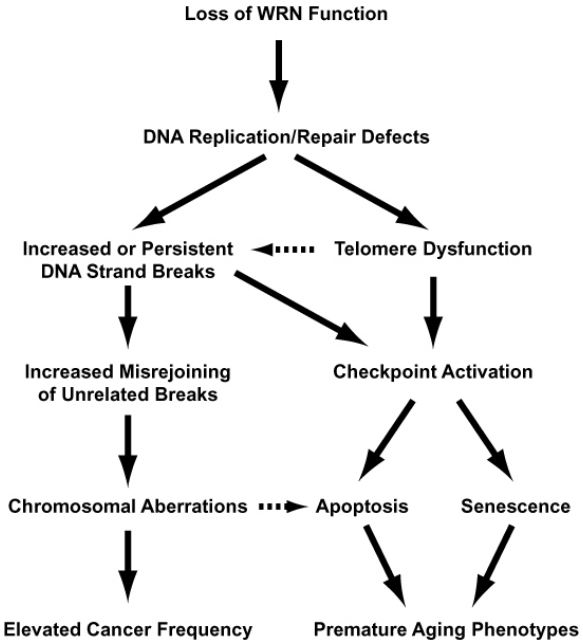

Figure 2. Loss of WRN function and downstream effects. Schematic diagram for how loss of WRN function in DNA metabolism potentially results in the elevated cancer frequency and early onset of other aging phenotypes associated with Werner syndrome.

replication blockage, and perhaps other pathways responding to DNA damage. It is certainly possible that these functions are independent, and loss of WRN function would cause overall genomic instability and telomere-specific abnormalities. Alternatively, WRN may play a single role in DNA metabolism that, when lost, has an acute effect in telomeric regions due to their highly repetitive nature. It seems possible that, within repetitive regions of DNA, recombinational repair pathways may be particularly subject to alignment or resolution problems that may lead to increased insertion or deletion events. By this reasoning, both the overall genome instability and the telomere-related changes might be due to a single defect that leads to increased recombination or the improper resolution of a specific recombinational repair pathway. These and other specific questions regarding the functions of WRN in DNA metabolism are yet to be elucidated.

It seems clear that genomic instability caused by WRN deficiency is responsible for the increased cancer incidence in WS (Figure 2). However, it remains to be determined why mesenchymal tumor types such as soft tissue sarcomas and osteosarcomas are disproportionately associated with loss of WRN function. In regard to this issue, it may be noteworthy that soft tissue sarcomas and osteosarcomas often use the ALT pathway to maintain telomere length instead of reactivating telomerase (145). This suggests that mechanisms related to telomere maintenance that are specifically operable in cells of mesenchymal origin could impact tumor initiation and progression. In one scenario, ALT pathways may be highly active in cells of mesenchymal origin. If, as mentioned above, WRN functions to suppress or properly resolve telomeric recombination events, a WRN deficiency may lead to higher rates of genomic instability due to aberrant telomeric recombination or more frequent telomere deprotection in mesenchymal cell types that result in higher relative appearance of mesenchymal tumors. In an alternative scenario, telomerase may be more highly repressed in mesenchymal cells than in other cell types. Although this might tend to suppress tumorigenesis overall, perhaps this also results more often in use of ALT to maintain telomeres and, when combined with a loss of WRN function, a similar sequence of events as outlined for the former mechanism.

The study of WS has already provided and will continue to produce mechanistic insights into the relationship between genomic instability and the accelerated development of age-related phenotypes associated with the syndrome. These same mechanisms may also contribute, albeit later, to development of aging characteristics in normal individuals. The acceleration of aging phenotypes other than cancer in WS can be most likely explained by DNA metabolic problems that activate checkpoints resulting in apoptosis or senescence (Figure 2). Over time, apoptotic cell death or gradual accumulation of non-functional senescent cells could either directly diminish tissue function or drain the pool of replicating cells and thus weaken the proliferative and regenerative capacity of certain tissues, particularly those that require sustained proliferation over lifetime for proper function. Although apoptosis is clearly protective against tumorigenesis, it is widely believed to play a key role in the development of many aging characteristics. However, apoptosis may be initiated by DNA-independent mechanisms as well as by DNA-mediated events. Although senescence and apoptosis due to telomeric dysfunction or genome-wide DNA metabolic problems apparently result in accelerated development of specific aging characteristics in WS, it is unclear whether DNA-mediated apoptosis and senescence play roles in normal aging. Further research is needed to determine whether and to what extent genomic instability and telomere-related mechanisms contribute to specific aging phenotypes in normal individuals.

7. ACKNOWLEDGEMENTS

Although all the excellent work on WS could not be cited here due to space limitations, the author would like to acknowledge every scientist who has studied Werner syndrome and RecQ helicases, with particular thanks to George Martin for his pioneering leadership in this area of research and to Vilhelm Bohr for convincing me to investigate this fascinating subject. The author also thanks Dr. Amrita Machwe for critical reading of the manuscript and for her continuous contributions to our research efforts over the past ten years.

8. REFERENCES

1. O. Werner: Uber katarakt in verbindung mit sklerodermie. *Doctoral dissertation*, Kiel University, Schmidt and Klauning (1904)

2. M. Goto: Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. *Mech. Ageing Dev.* 98(3), 239-254 (1997)

3. G. M. Martin and J. Oshima: Lessons from human progeroid syndromes. *Nature* 408(6809), 263-266 (2000)

4. M. Goto, M. Rubenstein, J. Weber, K. Woods and D. Drayna: Genetic linkage of Werner's syndrome to five markers on chromosome 8. *Nature* 355(6362), 735-738 (1992)

5. C.-E. Yu, J. Oshima, Y. H. Fu, E. M. Wijsman, F. Hisama, R. Alisch, S. Matthews, J. Nakura, T. Miki, S. Ouais, G. M. Martin, J. Mulligan and G. D. Schellenberg: Positional cloning of the Werner's syndrome gene. *Science* 272(5259), 258-262 (1996)

6. J. Oshima, C.-E. Yu, C. Piussan, G. Klein, J. Jabkowski, S. Balci, T. Miki, J. Nakura, T. Ogihara, J. Ells, M. Smith, M. I. Melaragno, M. Fraccaro, S. Scappaticci, J. Matthews, S. Ouais, A. Jarzebowicz, G. D. Schellenberg and G. M. Martin: Homozygous and compound heterozygous mutations at the Werner syndrome locus. *Hum. Mol. Genet.* 5(12), 1909-1913 (1996)

7. C.-E. Yu, J. Oshima, E. M. Wijsman, J. Nakura, T. Miki, C. Piussan, S. Matthews, Y. H. Fu, J. Mulligan, G. M. Martin and G. D. Schellenberg: Mutations in the consensus helicase domains of the Werner syndrome gene. *Am. J. Hum. Genet.* 60(2), 330-341 (1997)

8. T. Matsumoto, A. Shimamoto, M. Goto and Y. Furuichi: Impaired nuclear localization of defective DNA helicases in Werner's syndrome. *Nat. Genet.* 16(4), 335-336 (1997)

9. R. A. Marciniak, D. B. Lombard, F. B. Johnson and L. Guarente: Nucleolar localization of the Werner syndrome protein in human cells. *Proc. Natl. Acad. Sci. USA* 95(12), 6887-6892 (1998)

10. C. E. Ogburn, J. Oshima, M. Poot, R. Chen, K. E. Hunt, K. A. Gollahon, P. S. Rabinovitch and G. M. Martin: An apoptosis-inducing genotoxin differentiates heterozygotic carriers for Werner helicase mutations from wild-type and homozygous mutants. *Hum Genet.* 101(2), 121-125 (1997)

11. L. Ye, T. Miki, J. Nakura, J. Oshima, K. Kamino, H. Rakugi, H. Ikegami, J. Higaki, S. D. Edland, G. M. Martin and T. Ogihara: Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. *Am. J. Hum. Genet.* 68(4), 494-498 (1997)

12. N. Ogata, M. Shiraki, T. Hosoi, Y. Koshizuka, K. Nakamura and H. Kawaguichi: A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. *J. Bone Mineral Metab.* 19(5), 296-301 (2001)

13. V. A. Bohr, E. J. Metter, J. A. Harrigan, C. von Kobbe, J. L. Liu, M. D. Gray, A. Majumdar, D. M. Wilson III and M. M. Seidman: Werner syndrome protein 1367 variants and disposition towards coronary artery disease in Caucasian patients. *Mech. Ageing Dev.* 125(7), 491-496 (2004)

14. N. A. Ellis, J. Groden, T.-Z. Ye, J. Straughen, D. J. Lennon, S. Ciocci, M. Proytcheva and J. German: The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83(4), 655-666 (1995)

15. S. Kitao, A. Shimamoto, M. Goto, R. W. Miller, W. A. Smithson, N. M. Lindor and Y. Furuichi: Mutations in REQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* 22(1), 82-84 (1999)

16. H. A. Siitonen, O. Kopra, H. Kaariainen, H. Haravuori, R. M. Winter, A. M. Saamanen, L. Peltonen and M. Kestila: Molecular defect of the RAPADILINO syndrome expands the phenotype spectrum of REQL diseases. *Hum. Mol. Genet.* 12(21), 2837-2844 (2003)

17. L. van Maldergem, H. A. Siitonen, N. Jalkh, E. Chouery, M. de Roy, V. Delague, M. Muenke, E. W. Jabs, J. L. Cai, L. L. Wang, S. E. Plon, C. Fourneau, M. Kestila, Y. Gillerot, A. Megarbane and A. Verloes: Revisiting the craniosynostosis-radial ray hypoplasia association: Baller-Gerold syndrome caused by mutations in the RECQL4 gene. *J. Med. Genet.* 43(2), 148-152 (2006)

18. D. A. Bernstein, M. C. Zittel and J. L. Keck: High-resolution structure of the E. coli RecQ helicase catalytic core. *EMBO J.* 22(19), 4910-4921 (2003)

19. Z. Liu, M. J. Macias, M. J. Bottomley, G. Stier, J. P Linge, M. Nilges, P. Bork and M. Sattler: The three-dimensional structure of the HRDC domain and implications for the Werner and Bloom syndrome proteins. *Structure* 7(12), 1557-1566 (1999)

20. C. von Kobbe, N. H. Thoma, B. K. Czyzewski, N. P. Pavletich and V. A. Bohr: Werner syndrome protein contains three structure-specific DNA binding domains. *J. Biol. Chem.* 278(52), 52997-53006 (2003)

21. J.-S. Hu, H. Feng, W. Zeng, G.-X. Lin and X.-G. Xi: Solution structure of a multifunctional DNA- and protein-binding motif of human Werner syndrome protein. *Proc. Natl. Acad. Sci. USA* 102(51), 18379-18384 (2005)

22. J. W. Lee, R. Kusumoto, K. M. Doherty, G.-X. Lin, W. Zeng, W.-H. Cheng, C. von Kobbe, R. M. Brosh, Jr, J.-S. Hu and V. A. Bohr: Modulation of Werner syndrome protein function by a single mutation in the conserved RecQ domain. *J. Biol. Chem.* 280(47), 39627-39636 (2005)

23. I. S. Mian: Comparative sequence analysis of ribonucleases HII, III, II, PH and D. *Nucleic Acids Res.* 25(16), 3187-3195 (1997)

24. M. Lebel and P. Leder: A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors

of topoisomerase and loss of cellular proliferative capacity. *Proc. Natl. Acad. Sci. USA* 95(22), 13097-13102 (1998)

25. H. Yan, C.-Y. Chen, R. Kobayashi and J. Newport: Replication focus-forming activity 1 and the Werner syndrome gene product. *Nat. Genet.* 19(4), 375-378 (1998)

26. B. Li, N. Conway, S. Navarro, L. Comai and L. Comai: A conserved and species-specific functional interaction between the Werner syndrome-like exonuclease atWEX and the Ku heterodimer in Arabidopsis. *Nucleic Acids Res.* 33(21), 6861-6867 (2005)

27. M. D. Gray, J.-C. Shen, A. S. Kamath-Loeb, A. Blank, B. L. Sopher, G. M. Martin, J. Oshima and L. A. Loeb: The Werner syndrome protein is a DNA helicase. *Nat. Genet.* 17(1), 100-103 (1997)

28. A. S. Kamath-Loeb, P. Welcsh, M. Waite, E. T. Adman and L. A. Loeb: The enzymatic activities of the Werner syndrome protein are disabled by the amino acid polymorphism R834C. *J. Biol. Chem.* 279(53), 55499-55505 (2004)

29. D. K. Orren, R. M. Brosh, Jr., J. O. Nehlin, A. Machwe, M. D. Gray and V. A. Bohr: Enzymatic and DNA binding properties of purified WRN protein: high affinity binding to single-stranded DNA but not to DNA damaged by 4NQO. *Nucleic Acids Res.* 27(17), 3557-3566.

30. J.-C. Shen, M. D. Gray, J. Oshima and L. A. Loeb: Characterization of Werner syndrome protein DNA helicase activity: directionality, substrate dependence and stimulation by replication protein A. *Nucleic Acids Res.* 26(12), 2879-2885 (1998)

31. P. Mohaghegh, J. K. Karow, R. M. Brosh, Jr., V. A. Bohr and I. D. Hickson: The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. *Nucleic Acids Res.* 29(13), 2843-2849 (2001)

32. R. M. Brosh, Jr., A. Majumdar, S. Desai, I. D. Hickson, V. A. Bohr and M. M. Seidman: Unwinding of a DNA triple helix by the Werner and Bloom syndrome helicases. *J. Biol. Chem.* 276(5), 3024-3030 (2001)

33. R. M. Brosh, Jr., J. Waheed and J. A. Sommers: Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. *J. Biol. Chem.* 277(26), 23236-23245 (2002)

34. D. K. Orren, S. Theodore and A. Machwe: The Werner syndrome helicase/exonuclease (WRN) disrupts and degrades D-loops in vitro. *Biochemistry* 41(46), 13483-13488 (2002)

35. M. Fry and L. A. Loeb: Human Werner syndrome DNA helicase unwinds tetrahelical structures of the fragile X syndrome repeat sequences $d(CGG)_n$. *J. Biol. Chem.* 274(18), 12797-12802 (1999)

36. A. Constantinou, M. Tarsounas, J. K. Karow, R. M. Brosh, V. A. Bohr, I. D. Hickson and S. C. West: Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep.* 1(1), 80-84 (2000)

37. R. M. Brosh, Jr., D. K. Orren, J. O. Nehlin, P. H. Ravn, M. K. Kenny, A. Machwe and V. A. Bohr: Functional and physical interaction between WRN helicase and human replication protein A. *J. Biol. Chem.* 274(26), 18241-18350 (1999)

38. Q. Yang, R. Zhang, X. W. Wang, E. A. Spillare, S. P. Linke, D. Subramanian, J. D. Griffith, J. L. Li, I. D. Hickson, J. C. Shen, L. A. Loeb, S. J. Mazur, E. Appella, R. M. Brosh, Jr., P. Karmakar, V. A. Bohr and C. C. Harris: The processing of Holliday junctions by BLM and WRN helicases is regulated by p53. *J. Biol. Chem.* 277(35), 31980-31987 (2002)

39. P. L. Opresko, C. von Kobbe, J.-P. Laine, J. Harrigan, I. D. Hickson and V. A. Bohr: Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. *J. Biol. Chem.* 277(43), 41110-41119 (2002)

40. K. Baynton, M. Otterlei, M. Bjoras, C. von Kobbe, V. A. Bohr and E. Seeberg: WRN interacts physically and functionally with the recombination mediator protein RAD52. *J. Biol. Chem.* 278(38), 36476-36486

41. P. L. Opresko, P. A. Mason, E. R. Podell, M. Lei, I. D. Hickson, T. R. Cech and V. A. Bohr: POT1 stimulates RecQ helicases WRN and BLM to unwind telomeric DNA substrates. *J. Biol. Chem.* 280(37), 32069-32080 (2005)

42. A.. Machwe, L. Xiao, J. Groden, S. W. Matson and D. K. Orren: RecQ family members combine strand pairing and unwinding activities to catalyze strand exchange. *J. Biol. Chem.* 280(24), 23397-23407 (2005)

43. P. L. Garcia, Y. Liu, J Jiricny, S. C. West and P. Janscak: Human RECQ5β, a protein with DNA helicase and strand-annealing activities in a single polypeptide. *EMBO J.* 23(14), 2882-2891 (2004)

44. C. F. Cheok, L. Wu, P. L. Garcia, P. Janscak and I. D. Hickson: The Bloom's syndrome helicase promotes the annealing of complementary single-stranded DNA. *Nucleic Acids Res.* 33(12), 3932-3941 (2005)

45. S. Sharma, J. A. Sommers, S. Choudhary, J. K. Faulkner, S. Cui, L. Andreoli, L. Muzzolini, A. Vindigni and R. M. Brosh, Jr: Biochemical analysis of the DNA unwinding and strand annealing activities catalyzed by human RECQ1. *J. Biol. Chem.* 280(30), 28072-28084 (2005)

46. A. Machwe, E. M. Lozada, L. Xiao and D. K. Orren: Competition between the unwinding and strand pairing activities of the Werner and Bloom syndrome proteins. *BMC Mol. Biol.* 7(1), 1 (2006)

47. S. Huang, B. Li, M. D. Gray, J. Oshima, I. S. Mian and J. Campisi: The premature ageing syndrome protein, WRN, is a 3' → 5' exonuclease. *Nat. Genet.* 20(2), 114-116 (1998)

48. J.-C. Shen, M. D. Gray, J. Oshima, A. S. Kamath-Loeb, M. Fry and L. A. Loeb: Werner syndrome protein. I. DNA helicase and DNA exonuclease reside on the same polypeptide. *J. Biol. Chem.* 273(51), 34139-34144 (1998)

49. A. S. Kamath-Loeb, J.-C. Shen, L. A. Loeb and M. Fry: Werner syndrome protein. II. Characterization of the integral 3' → 5' DNA exonuclease. *J. Biol. Chem.* 273(51), 34145-34150 (1998)

50. J.-C. Shen and L. A. Loeb: Werner syndrome exonuclease catalyzes structure-dependent degradation of DNA. *Nucleic Acids Res.* 28(17), 3260-3268 (2000)

51. A. Machwe, L. Xiao, S. Theodore and D. K. Orren: DNase I footprinting and enhanced exonuclease function of the bipartite Werner syndrome protein (WRN) bound to partially melted duplex DNA. *J. Biol. Chem.* 277(6), 4492-4504 (2002)

52. S. Huang, S. Beresten, B. Li, J. Oshima, N.A. Ellis and J. Campisi: Characterization of the human and mouse WRN 3'→5' exonuclease. *Nucleic Acids Res.* 28(12), 2396-2405 (2000)

53. Y. Xue, G. C. Ratcliff, H. Wang, P. R. Davis-Searles, M. D. Gray, D. A. Erie and M. R. Redinbo: A minimal exonuclease domain of WRN forms a hexamer on DNA and possesses both 3'-5' exonuclease and 5'-protruding strand endonuclease activities. *Biochemistry* 41(9), 2901-2912 (2002)

54. M. P. Cooper, A. Machwe, D. K. Orren, D. Ramsden and V. A. Bohr: Ku complex interacts with and stimulates the Werner protein. *Genes Dev.* 14(8), 907-912 (2000)

55. B. Li and L. Comai: Functional interaction between Ku and the Werner syndrome protein in DNA end processing. *J. Biol. Chem.* 275(37), 28349-28352 (2000)

56. J. Friedemann, F. Grosse and S. Zhang: Nuclear DNA helicase II (RNA helicase A) interacts with Werner syndrome helicase and stimulates its exonuclease activity. *J. Biol. Chem.* 280(35), 31303-31313 (2005)

57. A. Machwe, R. Ganunis, V. A. Bohr and D. K. Orren. Selective blockage of the 3'→ 5' exonuclease activity of WRN protein by certain oxidative modifications and bulky lesions in DNA. *Nucleic Acids Res.* 28(14), 2762-2770 (2000)

58. D. K. Orren, A. Machwe, P. Karmakar, J. Piotrowski, M.P. Cooper and V.A. Bohr (2001). A functional interaction of Ku with Werner exonuclease facilitates digestion of damaged DNA. *Nucleic Acids Res.* 29(9), 1926-1934 (2001)

59. A. Machwe, L. Xiao and D. K. Orren: TRF2 recruits the Werner syndrome exonuclease for processing telomeric DNA. *Oncogene* 23(1), 149-156 (2004)

60. R. M. Brosh, Jr., P. Karmakar, J. A. Sommers, Q. Yang, X. W. Wang, E. A. Spillare, C. C. Harris and V. A. Bohr: p53 modulates the exonuclease activity of Werner syndrome protein. *J. Biol. Chem.* 276(37), 35093-35102 (2001)

61. C. von Kobbe, P. Karmakar, L. Dawut, P. Opresko, X. Zeng, R. M. Brosh, Jr., I. D. Hickson and V. A. Bohr: Colocalization, physical, and functional interaction between Werner and Bloom syndrome proteins. *J. Biol. Chem.* 277(24), 22035-22044 (2002)

62. A. Machwe, L. Xiao and D. K. Orren: Length-dependent degradation of single-stranded 3' ends by the Werner syndrome protein (WRN): implications for spatial orientation and coordinated 3' to 5' movement of its ATPase/helicase and exonuclease domains. *BMC Mol. Biol.* 7(1), 6 (2006)

63. D. A. Bernstein and J. L. Keck: Domain mapping of Escherichia coli RecQ defines the roles of conserved N- and C-terminal regions in the RecQ family. *Nucleic Acids Res.* 31(11), 2778-2785 (2003)

64. P. Janscak, P. L. Garcia, F. Hamburger, Y. Makuta, K. Shirashi, Y. Imai, H. Ikeda and T. A. Bickle: Characterization and mutational analysis of the RecQ core of the Bloom syndrome protein. *J. Mol. Biol.* 330(1), 29-42 (2003)

65. S. M. Yannone, S. Roy, D. W. Chen, M. B. Murphy, S. Huang, J. Campisi and D. J. Chen: Werner syndrome protein is regulated and phosphorylated by DNA-dependent protein kinase. *J. Biol. Chem.* 276(41), 38242-38248 (2001)

66. G. Blander, N. Zalle, Y. Daniely, J. Taplick, M. D. Gray and M. Oren: DNA damage-induced translocation of the Werner helicase is regulated by acetylation. *J. Biol. Chem.* 277(52), 50934-50940 (2002)

67. P. Karmakar, J. Piotrowski, R. M. Brosh, Jr., J. A. Sommers, S. P. Miller, W. H. Cheng, C. M. Snowden, D. A. Ramsden and V. A. Bohr: Werner protein is a target of DNA-dependent protein kinase in vivo and in vitro, and its catalytic activities are regulated by phosphorylation. *J. Biol. Chem.* 277(21), 18291-18302 (2002)

68. P. Pichierri, F. Rosselli and A. Franchitto: Werner's syndrome protein is phosphorylated in an ATR/ATM-dependent manner following replication arrest and DNA damage induced during the S phase of the cell cycle. *Oncogene* 22(10), 1491-1500 (2003)

69. P. Karmakar and V. A. Bohr: Cellular dynamics and modulation of WRN protein is DNA damage specific. *Mech. Ageing Dev.* 126(11), 1146-1158 (2005)

70. E. U. Kurz and S. P. Lees-Miller: DNA damage-induced activation of ATM and ATM-dependent signaling pathways. *DNA Repair* 3(8-9), 889-900 (2004)

71. S.-T. Kim, D.-S. Lim, C. E. Canman and M. B. Kastan: Substrate specificities and identification of putative substrates of ATM kinase family members. *J. Biol. Chem.* 274(53), 37538-37543 (1999)

72. Y. Kawabe, M. Seki, T. Seki, W.-S. Wang, O. Imamura, Y. Furuichi, H. Saitoh and T. Enomoto: Covalent modification of the Werner's syndrome gene product with the ubiquitin-related protein, SUMO-1. *J. Biol. Chem.* 275(28), 20963-20966 (2000)

73. M. D. Gray, L. Wang, H. Youssoufian, G. M. Martin and J. Oshima: Werner helicase is localized to transcriptionally active nucleoli of cycling cells. *Exp. Cell Res.* 242 (2), 487-494 (1998)

74. A. M. Rodriguez-Lopez, D. A. Jackson, F. Iborra and L. S. Cox: Asymmetry of DNA replication fork progression in Werner's syndrome. *Aging Cell* 1(1), 30-39 (2002)

75. A. M. Rodriguez-Lopez, D. A. Jackson, J. O. Nehlin, F. Iborra, A. V. Warren and L. S. Cox: Characterization of the interaction between WRN, the helicase/exonuclease defective in progeroid Werner's syndrome, and an essential replication factor, PCNA. *Mech. Ageing Dev.* 124(2), 167-174 (2003)

76. T. Suzuki, M. Shiratori, Y. Furuichi and T. Matsumoto: Diverged nuclear localization of Werner helicase in human and mouse cells. *Oncogene* 20(20), 2551-2558 (2001)

77. S. Sakamoto, K. Nishikawa, S.-J. Heo, M. Goto, Y. Furuichi and A. Shimamoto: Werner helicase relocates into nuclear foci in response to DNA damaging agents and co-localizes with RPA and Rad51. *Genes Cells* 6(5), 421-430 (2001)

78. A. Franchitto and P. Pichierri: Werner syndrome protein and the MRE11 complex are involved in a common pathway of replication fork recovery. *Cell Cycle* 3 (10), e78-e86 (2004)

79. W. H. Cheng, S. Sakamoto, J. T. Fox, K. Komatsu, J. Carney and V. A. Bohr: Werner syndrome protein associates with gamma H2AX in a manner that depends upon Nbs1. *FEBS Lett.* 579(6), 135-1356 (2005)

80. D. Salk, K. Au, H. Hoehn and G. M. Martin: Cytogenetics of Werner syndrome cultured skin fibroblasts: variegated translocation mosaicism. *Cytogenet. Cell Genet.* 30(2), 92-107 (1981)

81. E. Gebhart, R. Bauer, U. Raub, M. Schinzel, K. W. Ruprecht and J. B. Jonas: Spontaneous and induced chromosomal instability in Werner syndrome. *Hum. Genet.* 80(2), 135-139 (1988)

82. K.-I. Fukuchi, G. M. Martin and R. J. Monnat, Jr.: Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proc. Natl. Acad. Sci. USA* 86(15), 5893-5897 (1989)

83. R. Elli, L. Chessa, A. Antonelli, P. Petrinelli, R. Ambra and L. Marcucci: Effects of topoisomerase II inhibition in lymphoblasts from patients with progeroid and "chromosome instability" syndromes. *Cancer Genet. Cytogenet.* 87(2), 112-116 (1996)

84. P. Pichierri, A. Franchitto, P. Mosesso and F. Palitti: Werner's syndrome protein is required for correct recovery after replication arrest and DNA damage induced in S-phase of cell cycle. *Mol. Biol. Cell* 12(8), 2412-2421 (2001)

85. M. Okada, M. Goto, Y. Furuichi and M. Sugimoto: Differential effects of cytotoxic drugs on mortal and immortalized B-lymphoblastoid cell lines from normal and Werner's syndrome patients. *Biol. Pharm. Bull.* 21(3), 235-239 (1998)

86. M. Poot, K.A. Gollahon and P. S. Rabinovitch: Werner syndrome lymphoblastoid cells are sensitive to camptothecin-induced apoptosis in S-phase. *Hum. Genet.* 104(1), 10-14 (1999)

87. M. Poot, J. S. Yom, S. H. Whang, J. T. Kato, K. A. Gollahon and P. S. Rabinovitch: Werner syndrome cells are sensitive to DNA cross-linking drugs. *FASEB J.* 15(7), 1224-1226 (2001)

88. A. Blank, M. S. Bobola, B. Gold, S. Varadarajan, D. D. Kolstoe, E. H. Meade, P. S. Rabinovitch, L. A. Loeb, and J. R. Silber: The Werner syndrome proteins confers resistance to the DNA lesions N3-methyladenine and O^6-methylguanine: implications for WRN function. *DNA Repair* 3(6), 629-638 (2004)

89. C. Swanson, Y. Saintigny, M. J. Emond and R. J. Monnat, Jr.: The Werner syndrome protein has separable recombination and survival functions. *DNA Repair* 3(5), 475-482 (2004)

90. Y. Fujiwara, T. Higashikawa and M. Tatsumi: A retarded rate of DNA replication and normal level of DNA repair in Werner's syndrome fibroblasts in culture. *J. Cell Physiol.* 92(3), 365-374 (1977)

91. Y. Saintigny, K. Makienko, C. Swanson, M. J. Emond and R. J. Monnat, Jr.: Homologous recombination resolution defect in Werner syndrome. *Mol. Cell. Biol.* 22(20), 6971-6978 (2002)

92. F. Takeuchi, F. Hanaoka, M. Goto, M. Yamada and T. Miyamoto: Prolongation of S phase and whole cell cycle in Werner's syndrome fibroblasts. *Exp. Gerontol.* 17(6), 473-480 (1982)

93. M. Poot, H. Hoehn, T. M. Runger and G. M. Martin: Impaired S-phase transit of Werner syndrome cells

expressed in lymphoblastoid cell lines. *Exp. Cell Res.* 202(2), 267-273 (1992)

94. F. Takeuchi, F. Hanaoka, M. Goto, I. Akaoka, T. Hori, M. Yamada and T. Miyamoto: Altered frequency of initiation sites of DNA replication in Werner's syndrome cells. *Hum. Genet.* 60(4), 365-368 (1982)

95. M. Lebel, E. A. Spillare, C. C. Harris and P. Leder: The Werner syndrome gene product co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I. *J. Biol. Chem.* 274(53), 37795-37799 (1999)

96. A. S. Kamath-Loeb, E. Johansson, P. M. J. Burgers and L. A. Loeb: Functional interaction between the Werner syndrome protein and DNA polymerase δ. *Proc. Natl. Acad. Sci. USA* 97(9), 4603-4608 (2000)

97. R. M. Brosh, Jr., C. von Kobbe, J. A. Sommers, P. Karmakar, P. L. Opresko, J. Piotrowski, I. Dianova, G. L. Dianov and V. A. Bohr: Werner syndrome protein interacts with human flap endonuclease I and stimulates its cleavage activity. *EMBO J.* 20(20), 5791-5801 (2001)

98. C.-Y. Chen, J. Graham and H. Yan: Evidence for a replication function of FFA-1, the Xenopus orthologue of Werner syndrome protein. *J. Cell Biol.* 152(5), 985-996 (2001)

99. T. Kawabe, N. Tsuyama, S. Kitao, K. Nishikawa, A. Shimamoto, M. Shiratori, T. Matsumoto, K. Anno, T. Sato, Y. Mitsui, M. Seki, T. Enomoto, M. Goto, N. A. Ellis, T. Ide, Y. Furuichi and M. Sugimoto: Differential regulation of human RecQ family helicases in cell transformation and cell cycle. *Oncogene* 19(41), 4764-4772 (2000)

100. T. M. Runger, C. Bauer, B. Dekant, K. Moller, P. Sobotta, C. Czerny, M. Poot and G. M. Martin: Hypermutable ligation of plasmid DNA ends in cells from patients with Werner syndrome. *J. Invest. Dermatol.* 102(1), 45-48 (1994)

101. J. Oshima, S. Huang, J. Campisi and R. H. Schiestl: Lack of WRN results in extensive deletion at nonhomologous joining ends. *Cancer Res.* 62(2), 547-551 (2002)

102. B. Li and L. Comai: Displacement of DNA-PKcs from DNA ends by the Werner syndrome protein. *Nucleic Acids Res.* 30(17), 3653-3661 (2002)

103. M. Lebel, J. Lavoie, I. Gaudreault, M. Bronsard and R. Drouin: Genetic cooperation between the Werner syndrome protein and poly(ADP-ribose) polymerase-1 in preventing chromatid breaks, complex chromosomal rearrangements, and cancer in mice. *Am. J. Pathol.* 162(5), 1559-1569 (2003)

104. C. von Kobbe, J. A. Harrigan, A. May, P. L. Opresko, L. Dawut, W.-H. Cheng, and V. A. Bohr: Central role for the Werner syndrome protein/poly(ADP-ribose) polymerase I complex in the poly(ADP-ribosyl)ation pathway after DNA damage. *Mol. Cell. Biol.* 23(23), 8601-8613 (2003)

105. B. Li, S. Navarro, N. Kasahara and L. Comai: Identification and biochemical characterization of a Werner's syndrome protein complex with Ku70/80 and poly(ADP-ribose) polymerase-1. *J. Biol. Chem.* 279(14), 13659-13667 (2004)

106. H. Nakayama: RecQ family helicases: roles as tumor suppressor proteins. *Oncogene* 21(58), 9008-9021 (2002)

107. C. Z. Bachrati and I. D. Hickson: RecQ helicases: suppressors of tumorigenesis and premature aging. *Biochem. J.* 374(Part 3), 577-606 (2003)

108. R. R. Khakkar, J. A. Cobb, L. Bjergbaek, I. D. Hickson and S. M Gasser: RecQ helicases: multiple roles in genome maintenance. *Trends Cell Biol.* 13(9), 493-501 (2003)

109. R. J. Bennett and J. L. Keck: Structure and function of RecQ DNA helicases. *Crit. Rev. Biochem. Mol. Biol.* 39(2), 79-97 (2004)

110. P.R. Prince, M.J. Emond and R. J. Monnat, Jr.: Loss of Werner syndrome protein function promotes aberrant recombination. *Genes Dev.* 15(8), 933-938 (2001)

111. N. Zhang, R. Kaur, X. Lu, X. Shen, L. Li and R. J. Legerski: The PSO4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *J. Biol. Chem.* 280(49), 40559-40567 (2005)

112. H. Yan, J. McCane, T. Toczylowski and C. Chen: Analysis of the Xenopus Werner syndrome protein in DNA double-strand break repair. *J. Cell Biol.* 171(2), 217-227 (2005)

113. J. E. Haber: DNA recombination: the replication connection. *Trends Biochem. Sci.* 24(7), 271-275 (1999)

114. S. C. Kowalczykowski: Initiation of genetic recombination and recombination-dependent replication. *Trends Biochem. Sci.* 25(4), 156-165 (2000)

115. B. Michel: Replication fork arrest and DNA recombination. *Trends Biochem. Sci.* 25(4), 173-178 (2000)

116. M. M. Cox: The non-mutagenic repair of broken replication forks via recombination. *Mutat. Res.* 510(1-2), 107-120 (2002)

117. P. McGlynn and R. G. Lloyd: Recombinational repair and restart of damaged replication forks. *Nat. Rev. Mol. Cell Biol.* 3(11), 859-870 (2002)

118. L. Hayflick and P. S. Moorhead: The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 25, 585-621 (1961)

119. J. R. Smith and O. M. Pereira-Smith: Replicative senescence: implications for in vivo aging and tumor suppression. *Science* 273(5271), 63-67 (1996)

120. J. Campisi: Replicative senescence: an old lives' tale? *Cell* 84, 497-500 (1996)

121. R. G. A. Faragher and D. Kipling: How might replicative senescence contribute to human ageing? *Bioessays* 20(12), 985-991 (1998)

122. G. M. Martin, C. A. Sprague and C. J. Epstein: Replicative life-span of cultivated human cells: effects of donor's age, tissue, and genotype. *Lab. Invest.* 23(1), 86-92 (1970)

123. A. Machwe, D. K. Orren and V. A. Bohr: Accelerated methylation of ribosomal RNA genes during the cellular senescence of Werner syndrome fibroblasts. *FASEB J.* 14(12), 1715-1724 (2000)

124. J. Oshima, J. Campisi, T. C. A. Tannock and G. M. Martin: Regulation of *c-fos* expression in senescing Werner syndrome fibroblasts differs from that observed in senescing fibroblasts from normal donors. *J. Cell Physiol.* 162(2), 277-283 (1995)

125. T. Davis, S. K. Singhrao, F. S. Wyllie, M. F. Haughton, P. J. Smith, M. Wiltshire, D. Wynford-Thomas, C. J. Jones, R. G. A. Faragher and D. Kipling: Telomere-based proliferative lifespan barriers in Werner syndrome fibroblasts involve both p53-dependent and p53-independent mechanisms. *J. Cell Sci.* 116(Part 7), 1349-1357 (2003)

126. F. S. Wyllie, C. J. Jones, J. W. Skinner, M. F. Haughton, C. Wallis, D. Wynford-Thomas, R. G. A. Faragher and D. Kipling: Telomerase prevents the accelerated cell ageing of Werner syndrome fibroblasts. *Nat. Genet.* 24(1), 16-17 (2000)

127. D. Choi, P. S. Whittier, J. Oshima and W. D. Funk: Telomerase expression prevents replicative senescence but does not fully reset mRNA expression patterns in Werner syndrome cell strains. *FASEB J.* 15(6), 1014-1020 (2001)

128. M. M. Ouelette, L. D. McDaniel, W. E. Wright, J. W. Shay and R. A. Schultz: The establishment of telomerase-immortalized cell lines representing human chromosomal instability syndromes. *Hum. Mol. Genet.* 9(3), 403-411 (2000)

129. D. M. Baird, T. Davis, J. Rowson, C. J. Jones and D. Kipling: Normal telomere erosion rates at the single cell level in Werner syndrome fibroblast cells. *Hum. Mol. Genet.* 13(14), 1515-1524 (2004)

130. J. Karlseder, D. Broccoli, Y. Dai, S. Hardy and T. de Lange: p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science* 283(5406), 132101325 (1999)

131. J. Karlseder, A. Smogorzewska and T. de Lange. Senescence caused by altered telomeric state, not telomere loss. *Science* 295(5564), 2446-2449 (2002)

132. R.C. Allsopp, H. Vaziri, C. Patterson, S. Goldstein, E. V. Younglai, A.B. Futcher, C. W. Greider and C. B. Harley: Telomere length predicts the replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci. USA* 89(21), 10114-10118 (1992)

133. V. P. Schulz, V. A. Zakian, C. E. Ogburn, J. McKay, A. A. Jarzebowicz, S. D. Edland and G. M. Martin: Accelerated loss of telomeric repeats may not explain accelerated replicative decline of Werner syndrome cells. *Hum. Genet.* 97(6), 750-754 (1996)

134. H. Tahara, Y. Tokutake, S. Maeda, H. Kataoka, T. Watanabe, M. Satoh, T. Matsumoto, M. Sugawara, T. Ide, M. Goto, Y. Furuichi and M. Sugimoto: Abnormal telomere dynamics of B-lymphoblastoid cell strains from Werner's syndrome patients transformed by Epstein-Barr virus. *Oncogene* 15(16), 1911-1920 (1997)

135. D. B. Lombard, C. Beard, B. Johnson, R. A. Marciniak, J. Dausman, R. Bronson, J. E. Buhlmann, R. Lipman, R. Curry, A. Sharpe, R. Jaenisch and L. Guarente: Mutations in the WRN gene in mice accelerate mortality in a p53-null background. *Mol. Cell. Biol.* 20(9), 3286-3291 (2000)

136. L. Wang, C. E. Ogburn, C. B. Ware, W. C. Ladiges, H. Youssoufian, G. M. Martin and J. Oshima: Cellular Werner phenotypes in mice expressing a putative dominant-negative human WRN gene. *Genetics* 154(1), 357-362 (2000)

137. H. W. Lee, M. A. Blasco, G. J., Gottlieb, J. W Horner, C. W. Greider and R. A. DePinho: Essential role of mouse telomerase in highly proliferative organs. *Nature* 392(6676), 569-574 (1998)

138. S. Chang, A. S. Multani, N. G. Cabrera, M. L. Naylor, P. Laud, D. Lombard, S. Pathak, L. Guarente and R. A. DePinho: Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat. Genet. 36*(8), 877-882 (2004)

139. X. Du, J. Shen, N. Kugan, E.E. Furth, D.B. Lombard, C. Cheung, S. Pak, G. Luo, R.J. Pignolo, R.A. DePinho, L. Guarente and F.B. Johnson: Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. *Mol. Cell. Biol.* 24(19), 8437-8446 (2004)

140. P.R. Laud, A.S. Multani, S.M. Bailey, L.Wu, J. Ma, C. Kingsley, M. Lebel, S. Pathak, R.A. DePinho and S. Chang: Elevated telomere-telomere recombination in WRN-deficient, telomere dysfunctional cells promotes

escape from senescence and engagement of the ALT pathway. *Genes Dev.* 19(21), 2560-2570 (2005)

141. F. B. Johnson, R. A. Marciniak, M. McVey, S. A. Stewart, W. C. Hahn and L. Guarente: The Saccharomyces cerevisiae WRN homolog Sgs1p participates in telomere maintenance in cells lacking telomerase. *EMBO J.* 20(4), 905-913 (2001)

142. P. L. Opresko, M. Otterlei, J. Graakjaer, P. Bruheim, L. Dawut, S. Kolvraa, A. May, M. M. Seidman and V. A. Bohr: The Werner syndrome helicase and exonuclease cooperate to resolve telomeric D loops in a manner regulated by TRF1 and TRF2. *Mol. Cell* 14(6), 763-774 (2004)

143. L. Crabbe, R. E. Verdun, C. I. Haggblom and J. Karlseder: Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306(5703), 1951-1953 (2004)

144. Y. Bai and J. P. Murnane: Telomere instability in a human tumor cell line expressing a dominant-negative WRN protein. *Hum. Genet.* 113(4), 337-347 (2003)

145. A. Muntoni and R. R. Reddel: The first molecular details of ALT in human tumor cells. *Hum. Mol. Genet.* 14(special no. 2), R191-R196 (2005)

146. T. Tsurimoto, A. Shinozaki, M. Yano, M. Seki and T. Enomoto: Human Werner helicase interacting protein 1 (WRNIP1) functions as a novel modulator for DNA polymerase delta. *Genes Cells* 10(1), 13-22 (2005)

Key Words: Werner syndrome, RecQ helicases, Genomic Instability, Telomeres, Senescence, Apoptosis, Cancer, Aging, Review

Send correspondence to : David K. Orren, Ph.D., Graduate Center for Toxicology, University of Kentucky College of Medicine, 356 HSRB, 800 Rose Street, Lexington, Kentucky 40536-0305, Tel: 859-323-3612, Fax: 859-323-1059, E-mail: dkorre2@uky.edu