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Progress paper

The Werner syndrome protein at the crossroads of DNA repair and apoptosis

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Abstract

Werner syndrome (WS) is a premature aging disease characterized by genetic instability. WS is caused by mutations in a gene encoding for a 160 kDa nuclear protein, the Werner syndrome protein (WRN), which has exonuclease and helicase activities. The mechanism whereby WRN controls genome stability and life span is not known. Over the last few years, WRN has become the focus of intense investigation by a growing number of scientists. The studies carried out by many laboratories have provided a wealth of new information about the functional properties of WRN and its cellular partners. This review focuses on recent findings that demonstrate a functional interaction between WRN and two factors that bind to DNA breaks, Ku and poly(ADP-ribose) polymerase 1, and discuss how these interactions can influence fundamental cellular processes such as DNA repair, apoptosis and possibly regulate cell senescence and organismal aging.

Keywords: Werner syndrome; DNA repair; Apoptosis; Aging; DNA strand breaks.

Werner syndrome (WS) is an autosomal recessive premature aging disorder associated with a predisposition to cancer and cardiovascular disease (Epstein et al., 1966; Goto, 1997; Dyer and Sinclair, 1998). The first signs of this disorder appear after puberty and the disease is usually diagnosed in individuals 20–30 years of age. WS individuals show increased predisposition to diseases observed during normal aging such as arteriosclerosis, osteoporosis, type II diabetes mellitus and a variety of tumors, primarily of mesenchymal origin (Epstein et al., 1966; Goto et al., 1996). Myocardial infarction (MI) and cancer are the most common causes of death among WS patients, with a median age of death of approximately 47 years (Goto, 1997). The majority of WS cases have been linked to mutations in a single gene, the Werner syndrome gene, which is located on chromosome 8 (Yu et al., 1996). In addition, there are a few cases of ''atypical WS'' that do not have mutations in the WS gene. Interestingly, a subset of these individuals have mutations in the lamin A/C gene (Chen et al., 2003b), a gene that is mutated in children with the rare premature aging disorder known as the Hutchinson–Gilford syndrome (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003).

The WS gene encodes a protein (Werner syndrome protein, WRN) with strong homology to a class of enzymes called the RecQ helicases (Yu et al., 1996; Karow et al., 2000) (Fig. 1). In humans, this family includes proteins such as the Bloom syndrome (BS) and the Rothmund–Thompson syndrome (RTS), whose germline mutations are responsible for diseases associated with genomic instability (Hickson, 2003). In contrast to other human members of the RecQ helicase family, the amino terminal region of WRN contains an exonuclease domain, which is highly homologous to the nuclease domain of *Escherichia coli* DNA polymerase I and ribonuclease D (RNAse D) (Moser et al., 1997). Helicase and exonuclease activities with a 3′ to 5′ directionality have been demonstrated in vitro using recombinant WRN (Gray et al., 1997; Suzuki et al., 1997, 1999; Huang et al., 1998, 2000; Kamath-Loeb et al., 1998; Shen et al., 1998a,b). Moreover, a number of studies have indicated that WRN can unwind and/or hydrolyze a number of distinctive DNA structures, such as duplex DNA, branched DNA structures,

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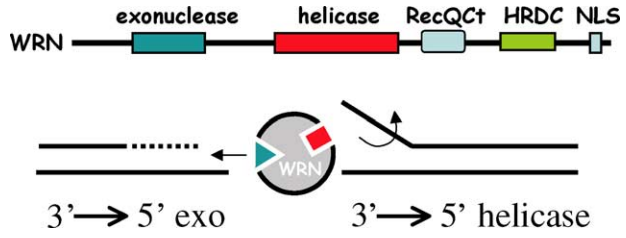

Fig. 1. Top panel: WRN domain structure (RecQCt: RecQ carboxy-terminal domain; HRDC: helicase and RNAse D carboxy-terminal domain; NLS: nuclear localization signal). Bottom panel: Diagram shows the directionality of WRN exonuclease and helicase activities.

D-loops, tetraplex DNA and four-way DNA junctions (Shen and Loeb, 2000; Mohaghegh et al., 2001; Brosh et al., 2002; Orren et al., 2002). The carboxy-terminal region of WRN contains two domains of unknown function that are conserved among the RecQ helicases and the RNAse D-like nucleases: a RecQ carboxy-terminal (RecQCt) domain and a helicase and RNAse D C-terminal (HRDC) domain. A nuclear localization signal is located at the carboxy-terminal end of WRN. All the WRN mutations that have been identified to date in WS patients are nonsense or frameshift mutations leading to truncated proteins missing the nuclear localization signal. The analysis of cell lines from WS patients shows in most cases no detectable WRN protein, suggesting that the truncated protein is rapidly degraded in the cytoplasm (Moser et al., 2000). There are however at least three patients diagnosed with WS in which a truncated protein has been detected by immunological methods (Goto et al., 1999).

Primary fibroblasts from WS individuals have a decreased life span and display genomic instability, which is marked by an elevated rate of chromosomal translocation and genomic deletions (Salk et al., 1985; Gebhart et al., 1988; Fukuchi et al., 1989). Significantly, cultured cells from WS patients are hypersensitive to a subset of DNA damaging agents (Ogburn et al., 1997; Gebhart et al., 1988; Okada et al., 1998; Poot et al., 1999, 2001, 2002; Hisama et al., 2000), suggesting that WRN may play a role in sensing DNA damage or in the repair of DNA lesions caused by these agents. WS cells display increased sensitivity to camptothecin (CPT). CPT is an antitumor drug that poisons topoisomerase I (topo I) by trapping topo I cleavage complexes on DNA (Liu et al., 2000). Exposure of cells to CPT therefore leads to the formation of DNA single-strand breaks (SSBs) that can then be converted to irreversible DNA double-strand breaks (DSBs) upon collision with an incoming replication fork (Pommier et al., 2003). Two forms of DNA repair, homologous recombination (HR) and non-homologous end joining (NHEJ), have been implicated in the repair of topo I-DNA adducts (Pommier et al., 2003). Interestingly, WRN interacts with topo I and stimulates its DNA relaxation activity (Lebel et al., 1999; Laine et al., 2003). WS cells are also hypersensitive to 4-nitroquinoline 1-oxide (4NQO) (Ogburn et al., 1997; Poot et al., 2002), a carcinogen that produces DNA strands breaks and bulky DNA adducts (Nagao and Sugimura, 1976). 4NQO-induced DNA damage can be repaired by several pathways including base excision repair (BER; SSBs), nucleotide excision repair (NER; DNA adducts) and NHEJ/HR (DSBs) (Hoeijmakers, 2001). Further studies have also indicated that WS cells display elevated sensitivity to agents that cause DNA interstrand crosslinks (ICLs) (Poot et al., 2001, 2002). These DNA lesions are thought to be repaired by a mechanism involving NER and HR (Dronkert and Kanaar, 2001). More recently, WRN-deficient cells have been shown to be hypersensitive to a site-specific alkylating agent (O^6-methylguanine) that can block DNA replication (Blank et al., 2004). Taken together, these studies do not identify a defect in a specific DNA repair pathway in WS cells. However, they suggest that WRN plays a key role in the cellular response to specific types of DNA damage.

Attempts to recapitulate the WS phenotype in mice have yielded limited information on WRN function. Mouse strains bearing a mutation in the carboxy-terminus of WRN that eliminates WRN expression do not show signs of premature aging, genomic instability or increased sensitivity to genotoxins (Lombard et al., 2000). Likewise, mice with a deletion of the WRN helicase domain have a normal life span (Lebel and Leder, 1998). Nevertheless, cells from these mice are hypersensitive to camptothecin, possibly indicative of an important role for the helicase activity in the detoxification of topo I-DNA adducts.

The identification of a variety of WRN-interacting proteins has provided insights into the possible physiological functions of WRN and a comprehensive description of these findings is found in recent reviews (Bohr et al., 2002; Chen and Oshima, 2002; Fry, 2002; Hickson, 2003; Opresko et al., 2003). In this commentary, we specifically focus on the potential function of WRN at DNA breaks, and reflect on how this protein, or lack thereof, may influence the cell's response to DNA damage.

The first evidence suggesting a link between WRN and the cellular apparatus that repairs DNA breaks was the discovery that WRN binds to and is recruited to DNA by Ku (Cooper et al., 2000; Li and Comai, 2000, 2001; Karmakar et al., 2002b). Ku is a heterodimeric factor composed of Ku70 and Ku80 (Ku86), which is involved in the repair of DSBs (Lieber et al., 2003) (Fig. 2A). A major implication of the finding that WRN interacts with Ku70/80 is that WRN may be involved in some aspects of DSB repair. DSBs are induced by a variety of genotoxic agents, including ionizing radiation and chemotherapeutic substances. The repair of DSBs occurs through the action of two major pathways, HR and NHEJ (Haber, 2000; Karran, 2000). Whereas repair by HR is mediated by the genes of the RAD52 epistasis group and is largely error-free, the NHEJ pathway catalyzes the error-prone rejoining of breaks and generally results in a loss or gain of genetic information. Both mechanisms operate in eukaryotic cells, however NHEJ is thought to be the prevalent DSBs repair pathway in higher eukaryotes. NHEJ is

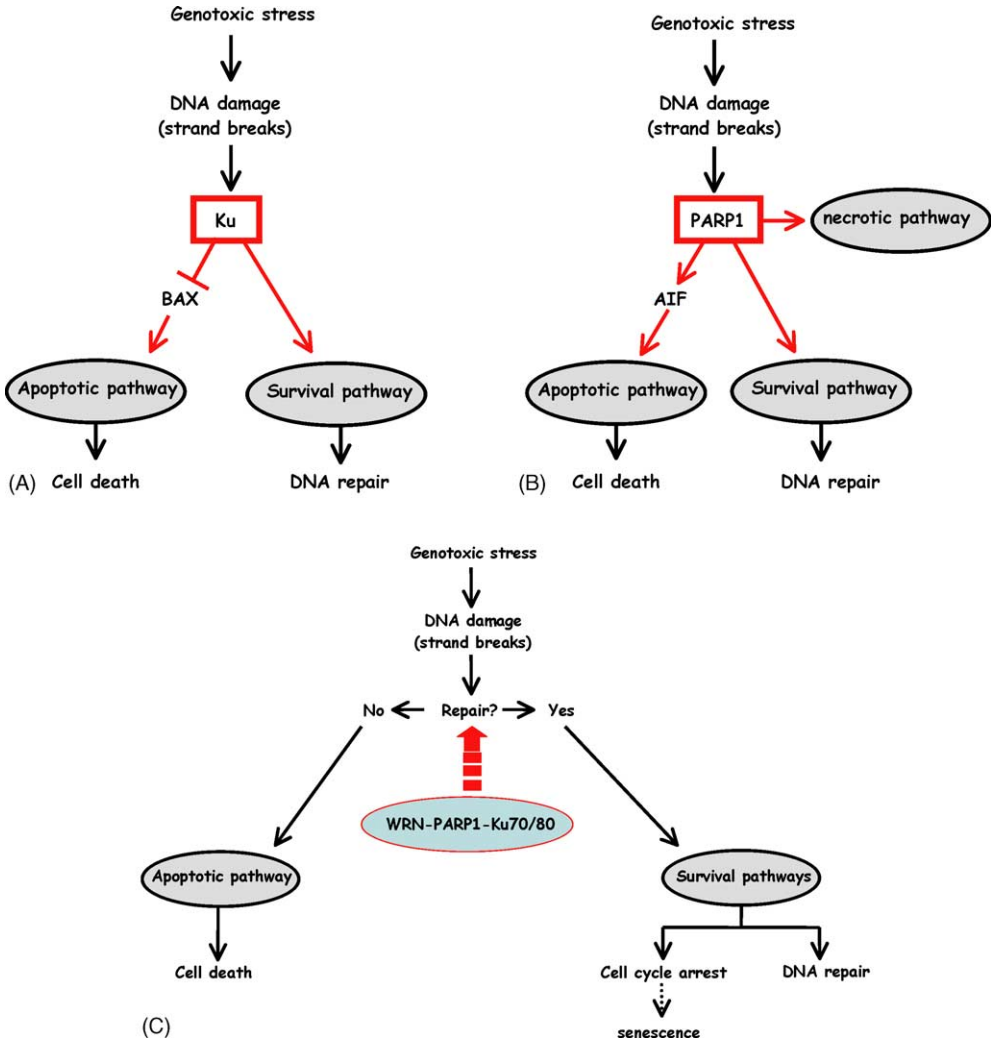

Fig. 2. (A) Involvement of Ku in pathways governing cell survival and apoptosis in response to DNA breaks. In response to DSBs Ku70/80 is recruited to DNA ends and functions in the NHEJ repair pathway. In addition, Ku70 directly controls cell survival and the activation of the apoptotic pathway by binding to and preventing the relocalization of Bax to the mitochondrial membrane. (B) Involvement of PARP-1 in pathways governing cell survival, apoptosis and necrosis in response to DNA breaks. PARP-1 binds to DNA strand breaks leading to the activation of its poly(ADP-ribosyl)ation activity. Modification of cellular factors by PARP-1 induces cell cycle arrest and allows DNA repair. In the instance of excessive DNA damage, sustained activation of PARP-1 results in the depletion of the NAD^+ pool and decreases ATP synthesis, which leads to the release of AIF from the mitochondria and activation of the caspase-independent apoptotic pathway or necrotic cell death. (C) Hypothetical model for the regulation of DNA repair and apoptosis by the WRN:Ku:PARP-1 complex. These proteins may control the cellular response to DNA strand breaks by regulating the activation of DNA repair (cell survival) or apoptotic (cell death) pathways (see text for details). The lack of any of these proteins may result in an abnormal response to specific genotoxic insults and lead to genomic instability, premature senescence or cell death.

carried out by a set of factors that include Ku70/80, the DNA-activated protein kinase (DNA-PKcs), ligase IV and XRCC4 (Lieber et al., 2003). Ku70/80 is the first factor that binds to the broken DNA ends and is thought to facilitate the recruitment of DNA-PKcs to DNA. Since in most instances the DNA ends may require trimming, it is likely that exonuclease or endonuclease activities will resect the DNA before the ends are covalently joined by the ligase IV–XRCC4 complex. The exo/endonuclease Artemis may fulfill this function, although other nucleases could also be involved in this process (Lieber et al., 2003). Importantly, any nucleolytic activity acting on the DNA end must be accurately controlled to prevent excessive degradation. Does the physical interaction between WRN and Ku suggest a possible function for WRN in DNA repair by NHEJ? Since WRN possesses an intrinsic exonuclease activity, it is tempting to speculate that this activity may be involved in the processing of DSBs before the ends are rejoined. Significantly, biochemical studies have indicated that Ku not only recruits WRN to DNA ends, but also stimulates and alters the properties of WRN exonuclease activity on a variety of DNA substrates (Li and Comai, 2000; Orren et al., 2001). Moreover, the WRN–Ku complex can effectively hydrolyze DNA molecules containing oxidized bases, such as 8-oxoguanine and 8-oxoadenine, which were previously shown to block WRN exonuclease activity (Machwe et al., 2000; Orren et al., 2001). Two independent studies have also indicated that DNA-PKcs phosphorylates WRN and

regulates its catalytic activity (Yannone et al., 2001; Karmakar et al., 2002a). However, there are conflicting results on whether the inhibition of WRN function by DNA-PKcs occurs by protein interaction (Yannone et al., 2001) or through phosphorylation (Karmakar et al., 2002a; Opresko et al., 2003).

Overall, these results suggest that synergistic activity between WRN and Ku/DNA-PKcs may be involved in the processing of broken DNA ends. However, these data do not provide unequivocal evidence that support the hypothesis that WRN plays a direct role in NHEJ. To further explore the link between WRN and NHEJ, the efficiency and fidelity of rejoining DNA ends was monitored by introducing linear plasmids with incompatible DNA ends into WS cells (Oshima et al., 2002; Chen et al., 2003a). These experiments show that the ligated DNA undergoes extensive deletions, suggesting that lack of WRN results in a hyperactive error-prone DNA end-joining pathway. Significantly, expression of the wild-type WRN prevents excessive DNA deletions. Yet, it is surprising that a WRN mutant defective in exonuclease and helicase activity was as proficient at complementation as the wild-type protein (Chen et al., 2003a). A possible interpretation of these data is that WRN, rather than actively participating in the NHEJ repair pathway, protects DNA from nucleolytic degradation and aberrant error-prone DNA repair. A role for WRN in regulating the activity of the NHEJ pathway is consistent with data demonstrating that WRN displaces DNA-PKcs from a DNA-bound Ku70/80 (Li and Comai, 2002). Based on this finding, one can envision that by preventing the recruitment of DNA-PKcs to the site of the DSB, WRN keeps the error-prone DNA repair machinery in check. Since it has been proposed that NHEJ competes with HR for the repair of DSBs (Haber, 2000; Allen et al., 2002, 2003), a corollary of this model would be that by precluding the assembly of a Ku70/80:DNA-PKcs complex at the site of DNA damage, WRN facilitates the recruitment of an error-free repair machinery, particularly during the S and G2 phases of the cell cycle when homologous DNA sequences are available. Indeed, a link between WRN and components of the HR repair pathway has been demonstrated by studies showing that WRN interacts with RAD52 (Baynton et al., 2003) and colocalizes with RAD51 in CPT-treated cells (Sakamoto et al., 2001). A potential role for WRN in the resolution of HR products and in the interplay between HR and NHEJ has been discussed in a recent article (Monnat and Saintigny, 2004). Alternatively, it is possible that the recruitment of WRN to DNA ends by Ku may be required for a subset of DNA breaks that need a specialized form of DNA processing before the two ends can be rejoined. For instance, the WRN–Ku complex may be recruited to DNA breaks adjacent to modified bases or DNA adducts, where the exonuclease activity of WRN is required to trim the DNA and remove the modified bases or DNA adducts before the DNA ends can be rejoined.

Clearly, the connection between WRN and NHEJ remains to be further examined, however the available data indicate an intimate link between WRN and components of the machinery that repairs DSBs. Does a potential function for WRN in the repair of DSBs explain the premature senescence of WS cells? It is still too early to say. However, it is remarkable that Ku80-null mice show accelerated aging and cells from these mice, as cell explanted from WS patients, display genomic instability and premature senescence (Vogel et al., 1999; DiFilippantonio et al., 2000; Ferguson et al., 2000). Moreover, recent data showing that senescing mammalian cells accumulate DSBs (Sedelnikova et al., 2004) and the repair of DSBs in older cells is associated with large DNA deletions (Seluanov et al., 2004) support a direct relationship between the aberrant repair of DSBs and cellular senescence. Thus, it is enticing to propose that lack of WRN may disrupt the regulatory processes controlling NHEJ and accelerate the age-associated decline in the fidelity of DSBs repair.

Whereas the importance of Ku in NHEJ has been firmly established for many years, interesting new data have revealed an unexpected role for Ku70 in Bax-mediated apoptosis. These studies have shown that cytoplasmic Ku70 is bound to the proapoptotic protein Bax and prevents the relocalization of Bax to the mitochondrial membrane (Sawada et al., 2003). Upon genotoxic stress, Bax is released from Ku70, relocalizes to the mitochondrial membrane and activates the caspase-dependent apoptotic pathway. The link between Ku and apoptosis as well as the relationship between nuclear Ku70/80 and cytoplasmic Ku70 need to be further analyzed. Such studies will certainly provide important insights into the many functions of this cellular factor. Whether WRN participates in the regulation of the cellular events that control the function of cytoplasmic Ku and Bax-mediated apoptotic cell death also remains to be determined.

The connection between WRN and the cellular factors that regulate DNA repair and apoptosis has been recently strengthened by the identification of a physical interaction between WRN and poly(ADP-ribose) polymerase-1 (PARP-1). Data from three laboratories have shown that PARP-1 binds to WRN and the RecQCt domain of WRN mediates the interaction between these two factors (Adelfalk et al., 2003; von Kobbe et al., 2003; Li et al., 2004). Chromatography studies have further indicated that WRN is bound to PARP-1 within a complex that contains Ku70/80 and in vitro binding assays have demonstrated that each of these factors make direct contacts with one another (Li et al., 2004). As Ku and PARP-1 are rather abundant factors, only a subpopulation of them is associated with WRN.

PARP-1 is a DNA-dependent poly(ADP-ribosyl)ating enzyme that binds to single and double-stranded DNA breaks (de Murcia and Menissier de Murcia, 1994; Muiras, 2003). PARP-1 catalyzes the addition of ADP-ribose polymers onto many target proteins when activated by DNA strand breaks, a process that may be necessary for the proper repair of damaged DNA (Fig. 2B). It is believed that the covalent attachment of long, negatively charged chains of

poly(ADP-ribose) facilitates the removal of target DNA-binding proteins such as histones from damaged DNA, a step that is probably required for the recruitment of repair factors to the site of the DNA lesion (D'Amours et al., 1999). Importantly, poly(ADP-ribose) polymerase activity has been linked to mammalian longevity (Burkle, 2001; Muiras, 2003). In addition to function in DNA repair, PARP-1 can induce apoptosis or necrosis in cells with extensive DNA damage. Sustained activation of PARP-1 induced by strand breaks initiates an energy consuming metabolic cycle that results in the depletion of dinucleotide pools and triggers the release of the mitochondrial apoptosis-inducing factor (AIF), an activator of the caspase-independent pathway of programmed cell death (Yu et al., 2002). Sustained activation of PARP-1 can also slow down the rate of glycolysis and mitochondrial respiration, which reduces ATP synthesis and may lead to necrotic cell death (Ha and Snyder, 1999).

Does PARP-1 affect WRN function? Biochemical analyses indicate that the interaction between WRN and PARP-1 does not directly influence WRN helicase or exonuclease activity (Li et al., 2004). On the other hand, PARP-1 poly(-ADP-ribosyl)ates Ku70/80 (Li et al., 2004). This covalent modification impairs the binding of this factor to DNA and results in a significant decrease of Ku-mediated stimulation of WRN exonuclease activity. These results suggest that PARP-1 may regulate the exonuclease activity of WRN once the WRN complex is recruited to DNA breaks. Interestingly, lack of WRN affects global poly(ADP-ribosyl)ation in response to a subset of DNA damaging agents, as a deficiency in poly(ADP-ribosyl)ation of cellular proteins has been observed in WS cells exposed to hydrogen peroxide and methyl methanesulfonate but not in WS cells treated with the radiomimetic agent bleomycin (von Kobbe et al., 2003). The reason for this deficiency remains to be elucidated, but it appears that the absence of a functional WRN prevents the activation of PARP-1 in response to DNA damage caused by oxidative stress and alkylating agents (von Kobbe et al., 2003). This may result in a poor activation of downstream DNA repair or apoptotic pathways and lead to the accumulation of cells with genetic abnormalities. A functional link between PARP-1 and WRN is further supported by genetic studies, which indicated that PARP-1 null/WRN helicase-deficient mice have higher incidence of cancer and fibroblasts from these mice display an increased frequency of chromosomal breaks (Lebel et al., 2003). Similarly, a functional interplay between PARP-1 and Ku is demonstrated by the analyses of PARP-1/Ku80 double-mutant mice. These mice die during embryogenesis and cultured blastocysts display an elevated level of apoptosis (Henrie et al., 2003; Tong et al., 2002). Moreover, Ku80 haploinsufficiency in PARP-1 null mice leads to the development of hepatocellular tumors bearing severe chromosomal abnormalities (Tong et al., 2002).

A link between WRN and apoptosis was first proposed by studies demonstrating that WRN fibroblasts exhibit a decreased p53-mediated apoptotic response (Spillare et al., 1999). p53 is a tumor suppressor necessary for maintaining cell growth control (Vogelstein et al., 2000; Ryan et al., 2001), which is activated in response to a number of stresses, including exposure to DNA damaging agents. The regulated interaction of p53 with cellular partners and the downstream events that follow p53 activation provide a network of signals that lead to cell cycle arrest, tumor suppression and apoptosis. p53 binds to WRN (Blander et al., 1999, 2000; Spillare et al., 1999) and inhibits its exonuclease activity (Brosh et al., 2001). Moreover, WRN is required for efficient induction of p53 (Blander et al., 2000). Biochemical and genetic analyses have also provided evidence for a functional link between p53 and PARP-1. PARP-1 binds to p53 and, through poly(ADP-ribosyl)ation, can regulate its transactivation activity (Wesierska-Gadek et al., 1996; Malanga et al., 1998; Wieler et al., 2003). Moreover, analysis of mice deficient in both p53 and PARP-1 show enhanced tumorigenesis and a marked increase in chromosomal abnormalities (Tong et al., 2001). A possible interpretation of these findings is that the interaction of p53 with WRN and PARP-1 modulates the biological effects of p53 in controlling cell growth and tumor suppression and any disruption of the delicate interplay between these factors could alter the cellular response to DNA damage.

The picture that emerges from all these studies is that WRN and its interacting partners may play a role in the surveillance of genome integrity and control the cell's response to genotoxic stress by facilitating the activation of the appropriate cellular pathways (Fig. 2C). If the DNA damage can be repaired, WRN and its associated factors may help recruiting the proper DNA repair factors to the site of the lesion. On the other hand, if the damage is excessive and repair can only have harmful consequences, the WRN complex may direct the activation of the apoptotic pathway. Lack of WRN may deregulate this monitoring system and result in anomalous activation of DNA repair or apoptosis in response to specific types of DNA damage, such a single and double-strand breaks. Aberrant DNA repair can cause genomic instability and lead to premature cell senescence or cancer. Likewise, even though apoptosis can be beneficial by eliminating defective cells, increased or aberrant activation of the apoptotic pathway may result in a critical reduction of cell numbers, which can lead to tissue atrophy and other aging-associated phenotypes in postmitotic tissues such as the heart and skeletal muscle. Thus, an imbalance in repair and survival caused by the lack of a functional WRN can have severe implications for organismal longevity.

In the last few years, a large number of biochemical and genetic studies have provided critical insights into the physiological functions of WRN. As there is much more to learn about the mechanisms leading to genomic instability in WS cells, it is important that future studies will continue to focus on the link between WRN and the cellular pathways that are activated by DNA strand breaks. Ultimately, such studies will provide mechanistic insights into the cellular processes that cause cell senescence and human aging.

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