The Heli-CASE of the Missing WRN Gene

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Summary

Werner Syndrome is an autosomal recessive disease characterized by genomic instability, accelerated telomere shortening, and premature aging. Also, Werner Syndrome patients experience increased cancer rates, believed to be directly related to the lack of interaction between the WRN gene and tumor suppressor gene p53. The WRN gene consists of three identical molecules and has both exonuclease and helicase activity, which work together in opposite directions. WRN has been shown to stimulate polymerase β, needed in DNA repair. WRN helicase activity can also bind and degrade G-quadruplexes, which inhibit transcription. Studies show that the tumor suppressor gene p53 co-localizes with WRN during the S phase, inhibiting the exonuclease activity of WRN, and dulling WRN's helicase ability to unwind Holliday Junctions; thus, revealing the regulation of WRN function by p53 is important for genomic stability. WRN also interacts with TRF2. TRF2 binds to DNA, attracts WRN, and stimulates the folding over of the 3' overhang of telomeres. Absence of WRN leads to telomere loss and chromosomal fusion which lead to genome instability, accumulation of mutations, and thus cancer. The lack of WRN leads to premature senescence and exhibition of clinical symptoms of aging, even though 91% of RNA pol II transcription genes are expressed similarly to normal aging cells. The purpose of this review is to summarize current literature concerning the molecular pathways of Werner Syndrome

Introduction

Werner Syndrome (WS) was discovered in 1903 by Otto Werner. As a student of ophthalmology in Germany, Werner noticed peculiar traits among four siblings who were around thirty years old (Blachford, 2002). The specific features he noted in these patients were gray hair, cataracts, hair loss, and skin abnormalities (Harvard, 2005). Other common pathogenic features of WS include diabetes mellitus, osteoporosis, atherosclerosis, systemic sclerosis, increased susceptibility to cancer, and other diseases generally associated with geriatric patients (Blachford, 2002).

Werner Syndrome is an autosomal recessive disease exhibiting symptoms of premature aging (Chang, 2004). The underlying cause of WS is the absence of the WRN gene which was discovered in 1996 by Yu et al. Since then, the Pathology Department of Washington University has been working diligently at identifying specific mutations of the WRN gene (University of Washington, 2005). Although the molecular basis of the disease is still unknown, the clinical symptoms and diagnosis of WS are fairly clear.

The onset of WS usually occurs between the ages of 30 and 40, however, it may be seen in patients as young as 20 (Blachford, 2002). Although the major onset does not occur until later, WS begins to affect patients when they hitpuberty, at which point they stop growing, which accounts for their small stature and low body mass, and begin to display other common features of the disease such as skin abnormalities (Blachford, 2002).

Other distinguishing pathological hallmarks of WS patients that are used for diagnosis are excessive hyaluronic acid in the urine; decreased fertility due to impaired gonad function and underdeveloped sex organs; and high bone density in the phalanges (in contrast with the onset of osteoporosis usually seen in WS patients). Calcium salt deposits are also seen, generally in the Achilles, elbow, and knee tendons (Blanchford, 2002).

Another very important feature of WS patients is their increased rate of uncommon cancers (Blanchford, 2002). Soft-tissue sarcoma and benign meningioma are two such cancers that are relatively prevalent in WS patients (Goto, 1996). The high incidence of cancer is believed to be directly related to the absence of interaction between the WRN gene and tumor suppressor, p53, in WS patients (Blander, 1999).

Although Werner Syndrome is not exclusive to any race, it is more common among Japanese people living in Japan because of the prevalence of interbreeding (Goto, 1996). It is estimated that one in 95,000 to 1,000,000 people are affected by WS, but it is difficult to estimate because the disease is not usually noticed until adolescence (Blachford, 2002). The diagnosis of WS is usually made based on the appearance of the three main complications associated with the disease which are cataracts, skin abnormalities, and small stature, followed by at least two more additional symptoms such as osteoperosis, heart problems, or cancer (Blanchford, 2002).

The prognosis of WS patients is fatal. Their life span is cut short by an average of 30 years, resulting in early death usually between the ages of 40 and 47. Most WS patients die as a result of heart complications, cancer, or stroke (Blanchford, 2002). There is currently no cure for WS. The only treatment that may be done is for patients' individual symptoms.

Although the molecular mechanisms underlying the WRN gene are still unknown, there have been several recent discoveries in the roles that WRN fulfills as it interacts with other proteins. Recent studies show that WRN is responsible for recruiting several proteins that are necessary for proper degradation of certain DNA structures, recognition of DNA damage, and the folding over of the 3' overhang of telomeres (Griffith, 1999; Opresko, 2002).

Premature Aging

Aging Basics

At the cellular level two primary phenotypes result from Werner syndrome—large deletions which cause mutations and short proliferative lifespan of cells closely associated with a mutation in WRN (Ostler et al., 2000). WRN has been found to interact with RPA, an enzyme that is required for DNA replication (Opresko et al., 2003).

Telomere loss

Aging is linked to telomere lose. In normal aging, each year telomeres shorten by 50 base pairs (Rhodes et al., 1995). Telomere lose can be corrected with the addition of telomerase which extends the telomeres. Several independent studies reported that telomerase in WS cells

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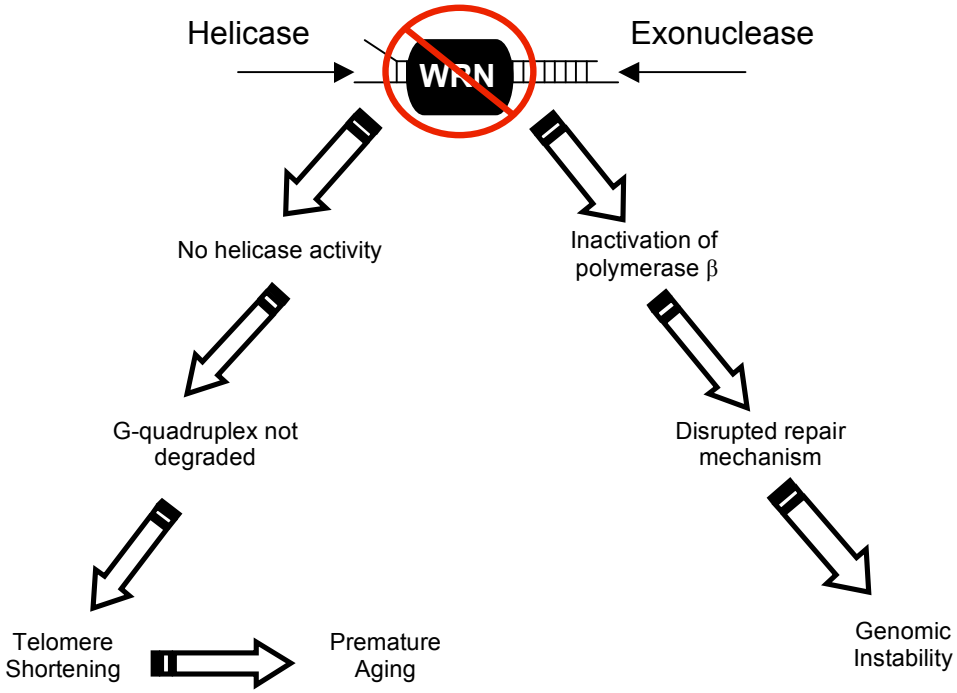

Figure 1: This figure depicts the line of events which occur due to the inhibition of helicase/exonuclease activity of WRN. The absence of WRN in Werner Syndrome patients prevents several interactions with proteins. These interactions are necessary for the degradation of transcription inhibiting structures and proper repair mechanisms. Absence of WRN leads to genome instability and characteristic phenotypic traits of WS patients.

increases the cellular life span (Opresko et al., 2003). This research supports the notation that premature senescence in WS cells is related to telomere dysfunction. Human telomerase halts the shortening of telomeres, preventing senescence in fibroblast, vascular endothelial cells and retinal pigmented epithelial cells (Ostler et al., 2000) More recently it was suggested that it is the alterations in telomere structure, as opposed to telomere length, that was responsible for prompting replicative senescence in cells of WS patients (Opresko et al., 2003).

Comparison of old genes, young genes and WS genes

WS phenotypes are secondary consequences of aberrant gene expression and a transcription defect may be crucial to the development of the syndrome (Kyng et al., 2003). Kyng et al. (2003) found that of the 6,912 RNA polymerase II transcription genes studied, only 6.3% varied when comparing WS or old gene donors to young gene donors. Ninety-one percent of annotated genes express similar expression changes. Only 3% were unique to WS, and 6% were unique to normal aging (Kyng et al., 2003). WS cells are deficient in RNA pol II transcription factors which are less expressed with age. "Correlating with the increased occurrence of cancers in old age and in WS, we found up-regulation of oncogenes (NDRG1, PIM1, RAB11A, and PIK3CA) and down-regulation of tumor suppressor genes (ST14, ST16, DKK3, BAP1)" (Kyng et al., 2003). These data support the characterization of WS showing the premature aging and increased occurrence of cancer as well as provide insight to the mechanisms by which these consequences occur.

WRN Gene

The WRN gene, inactive in Werner Syndrome patients, is among the RECQ-like family of helicases associated with hereditary disorders in humans (Huang et al., 2000). Of these helicases WRN is the only gene in this family known to have both helicase and exonuclease ability (Huang et. al 2000). The exonuclease domain of the WRN gene lies within the first 333 amino acids of a 1432 amino acid chain (Opresko et al., 2001), and purified alone has an apparent molecular weight of ~40 kDa (Huang et al., 2000). The full WRN gene weighed in at ~170 kDa (Huang et al., 2000).

WRN Helicase/Exonuclease Activity

The helicase and exonuclease of WRN act together on the same duplex (Opresko et al., 2001). The WRN helicase is active on the forked end of a DNA duplex where as the exonuclease activity is active on the blunt end of the duplex (Opresko et. al 2001). However, there seems to be no endonuclease activity and the exonuclease activity is limited to double stranded DNA with a 5' overhang as opposed to blunt end or single strand DNA (Huang et al., 2000). Exonuclease can also act on nicks or gaps in a DNA duplex. The exonuclease activity can successfully remove terminal mismatched nucleotides, but as the number of mismatches increase the exonuclease acts less efficiently. After a maximum of six terminal mismatched nucleotides, exonuclease activity was nearly undetectable (Huang et al., 2000). The termination of exonuclease activity would then result in an accumulation of mutations and thus cancer.

Aside from the nature of the duplex, the length also affected both activities of WRN; the exonuclease activity was greater on longer duplexes that were not unwound by the helicase, but there was less activity on shorter duplexes unwound by the helicase activity (Opresko et al., 2001). Because inactivation of the helicase activity showed a greater extent of exonuclease digestion, the WRN exonuclease activity seems to be regulated by the rapid unwinding of a duplex by the helicase activity.

It has been suggested that WRN binds to forked duplexes where the helicase starts unwinding at the forked

end and the exonuclease shortens the strand from the opposite end. The WRN will continue to bind to the duplex until the exonuclease digestion shortens the strand to an unstable length and the duplex dissolves (Huang et al., 2000). This suggests that when there are long segments of DNA that cannot be unwound by the helicase alone, both activities work together to remove the segments.

Oligomerization State of WRN

Although the oligomerization state of a helicase/exonuclease my not seem important, as we know structure equals function. WRN is a trimer. It is a complex made of three identical molecules, whereas many other helicases are hexamers including the BLM helicase associated with the related disease Blooms Syndrome. Because of this oligomerization state, it is possible for WRN to interact with other trimers such as Proliferating Cell Nuclear Antigen (PCNA). This interaction may be significant in halting the cell cycle and preventing proliferation.

WRN and DNA Repair

WRN interacts with a variety of proteins involved in DNA repair, for example, PCNA, RPA, and FEN-1(Harrigan et al., 2003). PCNA is part of a sliding clamp that forms a ring that maintains the connection of polymerase to its DNA template allowing uninterrupted synthesis (Cooper, 2000). WRN has been shown to have a direct interaction with PCNA *in vitro*, suggesting a unique role for WRN in DNA synthesis (Huang et al., 2000). RPA stimulates WRN helicase to unwind substrates that WRN cannot unwind alone (Harrigan et al., 2003). FEN-1 also physically interacts with WRN which stimulates its DNA flap cleavage activity (Harrigan et al., 2003). However, physical interaction does not give enough information to show a relationship relative to DNA synthesis or repair.

On the other hand, WRN has been found to physically interact with polymerase β (pol β), another protein involved in DNA repair. DNA polymerase β is essential for long and short patch base excision repair (BER), which is important in repairing DNA damaged by oxidized, alkylated, deaminated, and hydrolyzed bases (Harrigan et al., 2003). Within this physical interaction, pol β has no effect on WRN helicase or exonuclease activities, however, WRN has been shown to stimulate pol β DNA strand displacement synthesis (Harrigan et al., 2003). Because the presence of WRN increased the products of strand displacement, this suggests that WRN stimulates long patch BER by pol β (Harrigan et al., 2003).

MYC interaction with WRN

MYC oncoprotein coordinates both cell growth and division. In the cell with WRN, MYC stimulates transcription of the gene. WRN depletion from control fibroblasts led to rapid cellular senescence that could not be suppressed by hTERT expression (Grandori et al., 2003). The expression of hTERT would have been expected to result in the cell evading senescence. The increase of MYC in a cell increases the amount of WRN in the cell. Neither MYC nor WRN are differentiated cells and thus proliferate frequently. When TPA (tissue plasminogen activator) was added the leukemia cell lines, produced of MYC and WRN were down-regulated (Grandori et al., 2003).

WRN Helicase and Substrate Specificity

RecQ helicases, like WRN, have a common helicase domain which will bind and hydrolyze ATP. Two of these domains are the RecQ C-terminal region (RQC) and a helicase RNase D C-terminal (HRDC) domain (Opresko et al., 2004). These domains located on WRN helicase are known to bind to various DNA substrates and facilitate other DNA metabolic pathway steps (Opresko et al., 2004). The substrates explored, were blunt-end duplexes, tailed duplexes, 12nt bubbles, X-junctions, D-loops, and G-quadruplexes.

WRN and DNA duplexes

Substrate specificities of WRN helicase were tested on DNA blunt end duplexes of 25bp and 50bp. There were no measurable levels of DNA unwinding by WRN (Mohaghegh et al., 2001). Concentration levels of WRN helicase that actually unwound the blunt-end duplexes were much higher than in any other parts of the experiment where WRN successfully unwound substrates. It was also found that WRN does not have a high affinity for 3'- or 5'-tailed DNA duplexes when compared to other substrates tested (Mohaghegh et al., 2001). This suggests that WRN helicase activity is low to non-existent in the presence of DNA duplexes.

WRN and Synthetic X-junctions and Bubble Structures

Synthetic X-structures are models for the Holliday junction recombination intermediates (Li et al., 2001). These substrates were thus tested, because they could link WRN to playing a role in recombination. Experiments results indicate that WRN is able to unwind these junctions relatively well and 12 nucleotide bubble structures were unwound efficiently (Mohaghegh et al., 2001). These two findings when compared to the lack of unwinding of blunt-end duplexes possibly point to the conclusion that internal structural features are required in DNA to initiate unwinding (Mohaghegh et al., 2001).

WRN and D-loops

Another study was conducted to examine the interaction between WRN helicase and D-loop structures. The D-loop structure is the junction between the single- and double-stranded DNA and the available 3' end (Orren et al., 2002). D-loop substrates are thought to be an intermediate structure that forms during recombination pathways (Orren et al., 2002). They found that WRN does have a high affinity for binding and metabolizing D-loop structures. This may correlate with the idea that internal structural features are needed to initiate in unwinding (Mohaghegh et al., 2001). These results also indicate that both the helicase and exonuclease activities working together make this metabolic process more efficient (Orren et al., 2002).

WRN and G-quadruplexes

G-quadruplexes are guanine rich structures thought to play a role in processes like DNA replication, transcription, and recombination. The formation of G-quadruplexes is known to inhibit DNA replication (Li et al., 2001). Experimental evidences shows that WRN helicase is capable of degrading G-quadruplex substrates with very high affinity compared to all of the other substrates examined. This finding adds to the theory that an internal structural feature must be present to initiate unwinding. Without the helicase activity of WRN on the G-quadruplex and inhibition of DNA replication in WS patient cells, the 3' end is recognized as a mutation resulting in senescense.

Telomeres and Helicase Activity

Research on the pathogenesis of Werner Syndrome has recently been a prominent focus in biology. In particular, scientists have been focusing on the role of telomere structure and the presence or lack of helicase. Past research on the link between telomeric structure, helicase, and genomic instability have led to telomeres

posing as one of the main focal points in the search for the link between telomere length and pathogenic phenotypes (Chang et al., 2004).

The need for stable telomeres is an integral part of the body's defense against an accumulation of mutations that may lead to cancer. When the normal function of the telomere is disrupted and doomed to become shorter and shorter, as in Werner Syndrome patient cells, a likely result is cancer (Opresko et al., 2004).

Telomere Shortening and Fusion

The association of Werner Syndrome and telomere dysfunction has been established. Recent in vivo studies on a mouse model have shown that mice with long telomeres but lack the WRN gene do not display the phenotypic characteristics of WS. In their study, Chang et al., produced several generations of WRN deficient mice intercrossed with TERC deficient mice. This produced shorter and shorter telomeres through successive generations along with increased pathogenesis. Pathogenic phenotypes were not seen in the G5-G6 generations of $TERC^{-/-}$ $WRN^{+/+}$ mice as they were in $TERC^{-/-}$ $WRN^{-/-}$ of the same generation and age. The WRN deficient mice of the G5-G6 generation also expressed chromosomal fusion of the p-p arm and accelerated shortening of the telomeres (2004). Another similar study of the mouse model by, Du et al. (2005), provided results that agree with those of Chang et al. (2004). In addition, Du presented an idea based on the data showing that telomerase is able to compensate for some problems caused by helicase deficiency. The fact that WRN deficiency affects primarily cells that have slow replication rates and low levels of telomerase activity, suggest that the turnover rates are high enough to cause telomere shortening as they age. However, telomerase activity is too low to compensate for this loss and prevent telomere dysfunction (Du et al., 2005).

Cancer

The high incidence of cancer in WS patients is due to genomic instability. Although the exact pathways of WRN and the tumor suppressor protein p53 are not known, there is evidence to suggest that the interaction of the two has clear implications for the high incidence of cancer.

p53 is responsible for halting the cell cycle in both G phases so that damaged DNA may be repaired. Therefore, without p53 action there is increased genomic instability (Mohaghegh et al. 2001). The expression of p53 action is decreased in WS patient cells because p53 and WRN seem to have to interact for p53 to function properly (Opresko et al., 2004).

The speculated links between p53 expression, WRN, genomic instability, and cancer are thus logical. Without the WRN, p53 does not recognize damaged DNA that needs to be repaired. Lack of damage control leads to genomic instability and an accumulation of mutations, which result in cancer.

TRF2 and Helicase Activity

TRF2 is a protein that binds to and maintains telomeres throughout the cell cycle (Opresko et al., 2004). TRF2's importance lies in its function as a promoter for the helicase activity of WRN (Opresko et al., 2004). Therefore, with the absence of WRN in WS patients, TRF2 is unable to perform its duty and telomeric specific structures that need to be degraded remain. These structures then act as barriers against various transcription factors and telomeres are left without being fully transcribed. Therefore, the result is incomplete telomeres which leads to genomic instability and cancer.

TRF2 is believed to directly interact with the WRN protein. TRF2 first binds to the double-stranded telomere regions of DNA. WRN, which has a high affinity for TRF2, then binds to the TRF2 . The interaction between WRN and TRF2 may serve to stabilize TRF2 in its active form or to improve TRF2's interaction with DNA (Opresko et al., 2002). If the enzyme is able to bind to the telomere sequence and attract WRN, the nature of WRN to act as a helicase will prepare the telomere sequence for transcription.

Another important role that TRF2 is believed to play in telomere functionality involves t loops. T loops are the formations at the end of telomeres created when the 3' overhang bends back on itself to close the end of the DNA. TRF2 is believed to facilitate this folding over behavior (Griffith et al., 1999). Appropriate functioning of this mechanism is necessary for the capping of chromosomes.

In WS patients the WRN gene is absent. Because TRF2 must work with WRN to do its job, in the absence of WRN, TRF2 is inhibited. When TRF2 is inhibited, t loops do not form and the ends of the chromosomes are left uncapped. Inhibition of TRF2 also leads to the activation of a DNA damage checkpoint pathway. Therefore, when the incomplete (uncapped) telomere is checked it will be recognized as damaged DNA, leading to cessation of the cell cycle and apoptosis (Griffith et al., 1999).

WRN and Genomic Instability

A number of studies suggest that defects in the processes of DNA replication, repair, recombination, or a combination of these pathways are responsible for the genomic instability of WS (Brosh et al., 2002). The tumor suppressor gene p53 has been found to play a critical role in maintaining genomic integrity by interacting with WRN in two different ways (Brosh et al., 2001). The interaction between WRN and FEN-1, a DNA structure-specific nuclease, has also been found important in the maintenance of genomic stability (Brosh et al., 2002).

p53 and WRN Exonuclease Activity

To investigate the relationship between p53 and WRN, the effect of p53 protein on WRN exonuclease was tested. It was discovered that p53 effectively inhibits WRN exonuclease activity (Brosh et al., 2001). More specifically, p53 inhibits the exonuclease activity of the full-length WRN protein by binding the carboxyl-terminal region (Brosh et al., 2001). Thus the modulation of WRN exonuclease activity by p53 is dependent on protein interaction and likely to be influenced by the DNA binding properties of p53 (Brosh et al., 2001).

There are likely biological consequences of inhibiting WRN exonuclease activity. Brosh et al. (2001) found that WRN exits the nucleolus and colocalizes with p53 in the nucleoplasm when cells were arrested in S phase. This suggests that WRN and p53 may be associated with the DNA replication complex. The interaction of p53 and WRN might be important to direct S-phase cells into apoptosis (Brosh et al., 2001).

The weaken level of p53-mediated apoptosisin WS cells might be explained by the absence of WRN-p53 interaction (Brosh et al., 2001). This interaction might serve as a signal for programmed cell death. Suggesting that p53 modulation of WRN exonuclease activity may be critical for p53-mediated apoptosis.

p53 and WRN Helicase Activity

WRN unwinds Holliday junctions (HJ), reducing inappropriate DNA recombination (Yang et al., 2002).

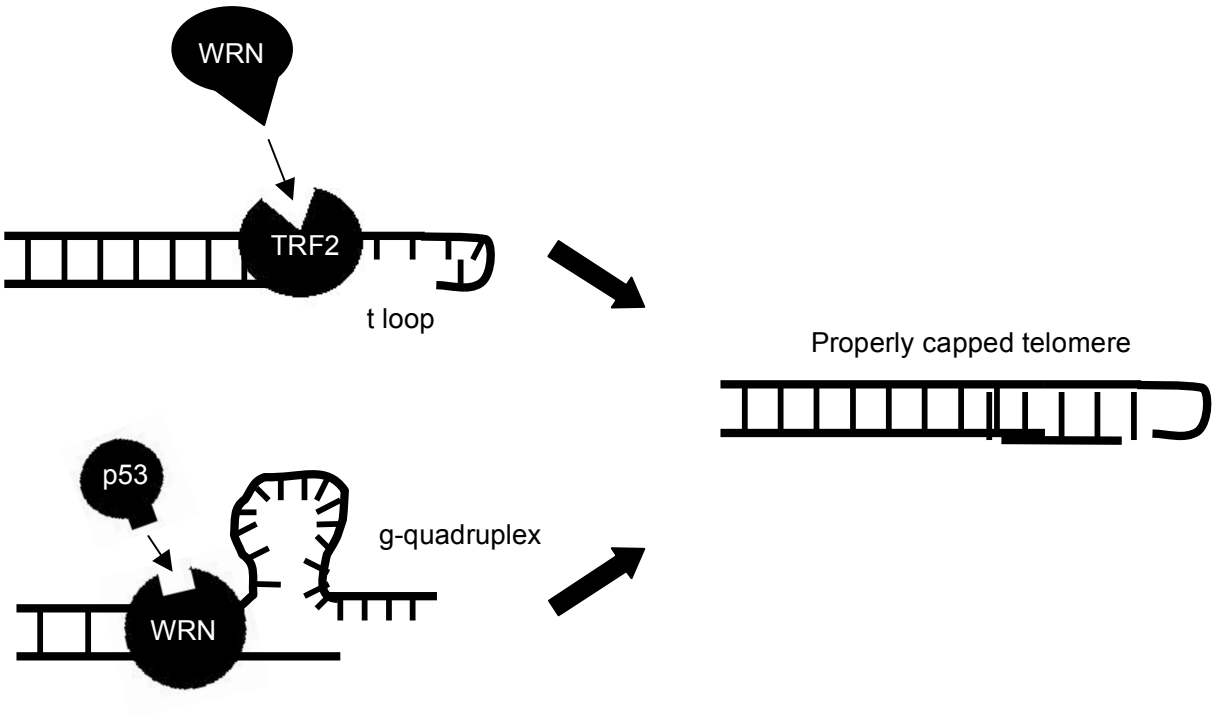

Figure 2: Proper transcription and capping of telomeres is essential for normal functioning of the telomere.
The colocalization of WRN with both TRF2 and p53 leads to proper capping of the telomere and its genetic stability. Absence of WRN, therefore lead to genetic instability and a heightened predisposition to cancer

Homologous recombination (HR) is an important process that repairs DNA damage that occurs during nearly every round of DNA replication. It was found that p53 modulates the ability of WRN helicase to disrupt HJ (Yang et al., 2002). It was also found that p53 phosphorylation, its inactive form, will decrease unmodified p53 inhibition of WRN helicase activity, allowing WRN to function properly (Yang et al., 2002). These data suggest that p53 plays a functional role in the helicase-HR pathway.

WRN and FEN-1

FEN-1 is a DNA structure-specific nuclease implicated in pathways of DNA metabolism that are important for genomic stability (Brosh et al., 2002). To understand the potential importance of the WRN – FEN-1 interaction in DNA replication, the effect of WRN on FEN-1 cleavage was tested. Brosh et al. (2002) found that WRN stimulates FEN-1 cleavage activity, but not when there is a fold-back flap structure. This study also showed that the ability of WRN to stimulate FEN-1 cleavage is optimal under conditions in which WRN does not unwind the 5' flap structure (Brosh et al., 2002). Stimulation of FEN-1 cleavage of 5' flaps may be important in Okazaki fragment processing during lagging strand synthesis at the replication fork (Brosh et al., 2002). Delayed action of FEN-1 cleavage of these fragments may be harmful due to the millions of them that get produced each time the genome is replicated. Defected Okazaki fragment processing causes double strand breaks which may lead to genomic instability in WS (Brosh et al., 2002).

Conclusion

Werner syndrome is an autosomal recessive disease that is characterized by premature aging and genomic instability. The gene thought responsible for the characteristics shown in Werner patients has both helicase and exonuclease activity. WRN helicase is highly substrate specific mainly for D-loop structures and G-quadruplexes suggesting that internal structures are required for DNA unwinding. The exonuclease activity works together with the helicase to unwind and remove terminal nucleotides. WRN also associates with repair proteins suggesting a unique role for the WRN gene in DNA repair. WRN is also associated with accelerated telomere loss and premature aging. Telomere shortening and chromosomal fusion form the basis of genomic instability that leads to cancer. The co-localization of the WRN gene and the telomere maintenance protein TRF-2 stimulate helicase activity.

WRN also co-localizes with p53 which inhibits exonuclease activity and dulls WRN helicase ability to unwind inappropriate DNA structures. The symptoms of aging and genomic instability that Werner Syndrome patients exhibit require the loss of function in the WRN gene and the WRN gene and the protein it produces have been found to be important in many factors of DNA maintenance,. If this gene were replaced or became functioning in WS patients, the chances of getting WS would be minimal. To further study this conclusion, experiments using a knockout WRN gene and an added normal functioning WRN gene are necessary. Because there have only been studies done *in vitro* with the WRN gene and its functioning in WS, the next step is to study the function of this important gene *in vivo*.

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