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CMLS Cellular and Molecular Life Sciences

Human Genome and Diseases: A new series of reviews in CMLS

The human genome sequence has been published in Nature (vol. 409, 15 Feb. 2001) and Science (vol. 291, 16 Feb. 2001). This breakthrough represents not an ending, but the beginning of a new approach to biology: it will more particularly revolutionize the way we look at human disease.

As a contribution in the field of biomedicine, CMLS will publish reviews from time to time dealing with developments concerning cellular and molecular aspects of genetic diseases. This series starts with Lebel's review on the Werner syndrome (see below).

Werner Syndrome: genetic and molecular basis of a premature aging disorder

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Abstract. Werner syndrome (WS) is a rare autosomal recessive disorder characterized by genomic instability and by the premature onset of a number of age-related diseases, including cancers. The gene responsible for WS encodes a protein that has an exonuclease domain and a domain similar to DNA helicases of the RecQ-like subfamily. Accumulating evidence indicates that the WS gene product is involved in resolving aberrant DNA structures that may arise during the process of DNA replication and transcription. Such processes generate regions of single-stranded DNA that may inadvertently provide a substrate for the initiation of recombination. Various mechanisms have evolved to ensure that recombination does not occur promiscuously during these events, and results are consistent with a model in which the WS protein is part of one such mechanism.

Key words. Werner Syndrome; premature aging; genomic instability; helicases; topoisomerases; DNA replication; transcription; recombination.

Introduction

WS was first described by Otto Werner in 1904 in a family displaying diseases that usually affect much older individuals. It was only 3–4 decades later that this disorder was catalogued as an inherited disease with several principal characteristics [review in 1]. Although there is variability in the phenotype from one patient to another, this syndrome is characterized by the early onset of age-related symptoms such as osteoporosis, diabetes mellitus, ocular cataracts, early graying of the hair, atherosclerosis and several types of neoplasms. The cause of death is either cancer or cardiovascular disease and occurs at a median age of 47 years [reviewed in 1]. WS also includes features not associated with aging, such as short stature, hyperkeratosis, subcutaneous atrophy, trophic ulcers of the legs, telangiectasia, calcification of the blood vessels, increased hyaluronic acid in the urine, hypogonadism and reduced fertility in both sexes. In addition, WS patients have a tendency to develop rare types of cancer [2].

Therefore, WS is phenotypically distinct from normal aging and is viewed more as a caricature of accelerated aging [reviewed in 3]. Nevertheless, the WS genetic defect does alter the rate of appearance of several important age-related symptoms, thus providing scientists with new insights into the molecular pathogenesis of these diseases.

WS is an uncommon autosomal recessive disorder with a low incidence in the general population (1/1,000,000). However, it is more common amongst Japanese, and the incidence of this syndrome rises to 1/3000 live birth in some prefectures in Japan due to consanguineous marriages [4, 5]. The genetic study of several Japanese families with WS permitted identification of the locus responsible for the disorder on chromosome 8 (at 8p12) [6, 7]. Based on these families, the gene (WRN) was identified by positional cloning and the complementary DNA (cDNA) was completely sequenced [8]. The predicted protein is 1432 amino acids in length and contains a domain with significant similarity to the *Escherichia coli* RecQ DNA helicase [9]. To date, more than 25 different mutations amongst WS patients have been identified throughout the 34 exons of the WRN gene [10, 11]. Mutations include stop codons, insertions, deletions and exon deletions [reviewed in 11]. Only one missense mutation has been recorded, from a French family with WS [12]. As a putative nuclear localization signal was identified near the end of the carboxy terminus of the WRN peptide [13], most of the mutations recorded to date would lead to the synthesis of a truncated peptide with impaired nuclear localization. However, Western blot analysis of several WS cell lines with an antibody against the human WRN peptide failed to detect any stable truncated mutant proteins [14, 15]. Thus, the mutations in the WS cell lines studied up to now resemble null mutations at the protein levels.

As indicated above, WS is a recessive disorder. However, there is evidence for higher rates of malignancy in heterozygotes as well. Moreover, a cellular phenotype different from wild-type siblings has been reported in heterozygotes [1, 2, 4, 16]. Again, as a stable mutant protein was not detected by Western blots in any WS cell lines studied to date, the phenotype encountered in heterozygous individuals may reflect haploid insufficiency.

One study has reported an association of a specific polymorphic WRN allele with myocardial infarction in the Japanese population [17]. However, a similar study on the Finnish population indicated no evidence of an association of this WRN polymorphism with longevity [18]. This contradiction in the data from both populations may be due to differing genetic background (ethnic variability). It may also reflect the differences in environmental factors that may affect the aging phenotype. In this respect, it is interesting to note that heterozygous carriers have an enhanced sensitivity to certain environmental genotoxic agents compared with wild-type individuals [19]. Thus, a deleterious phenotype associated with the heterozygotes could be of potential health concern in the general population [19]. More long-term correlative studies between age-related diseases and WS polymorphism or mutation are required to determine health risks.

Cellular phenotype of WS cells

At the cellular level, the phenotype of cultured fibroblasts explanted from patients suffering from WS also suggests a parallel between WS and aging. The proliferative life span of WS fibroblasts is reduced compared with age-matched controls. At first glance, WS cells behave similarly to fibroblasts established from elderly individuals which senesce in culture more rapidly than cells from young donors [20–22]. However, careful analysis of explanted wild-type and WS fibroblasts has indicated that the fraction of WS cells exiting the cell cycle during culturing increases more rapidly than the same fraction in cultured wild-type cells. Hence, WS cells have an enhanced rate of loss of proliferative capacity compared with wild-type cells [21].

It is known that replicative decline of somatic cells is associated with loss of telomeric repeats [reviewed in 23]. An acceleration of normal telomere-driven replicative senescence can also be detected in WS cells. However, several reports have indicated that WS cells may have an abnormal telomere dynamics in vitro, and they stop dividing with telomeres longer than normally seen in senescent wild-type cells [22, 24]. In addition, multiple changes in gene expression accompany cellular senescence. For example, c-fos expression is normally repressed in normal senescent fibroblasts. There is one study reporting that c-fos messenger RNA (mRNA) and protein inducibility are preserved in passaged WS cells, unlike senescent wild-type cells [25]. Thus, there are subtle differences between senescent wild-type and WS cells. Nonetheless, recent reports have shown that forced expression of the human catalytic subunit of telomerase in WS cells prevents replicative senescence [26, 27].

The reduction in replicative life span of WS cells may also be related in part to the increased genomic instability observed in these cells [reviewed in 28]. WS cells exhibit variegated chromosomal translocations and deletions. Such chromosomal abnormalities were found in vitro and in vivo in skin fibroblast cell lines as well as from lymphoblastoid cell lines made from circulating lymphocytes of WS patients [20, 29, 30]. Deletions in the telomeres of WS cells may affect overall telomeric distribution, which in turn can lead to replicative senescence.

Chromosomal aberrations may affect genes involved in different aspects of cellular metabolism, cell growth or

even cellular transformation. Mutations in the *HPRT* gene have been extensively studied in WS cells, and deletions appear to be a dominant type of mutation in primary or SV40-transformed WS cells [31–33]. The *HPRT* gene product is involved in the metabolic salvage pathway of nucleotides. It was found that the deletions resulted from illegitimate recombination between donor DNA that shares little nucleotide sequence identity [reviewed in 3]. Similarly, data on an unrelated gene (glycophorin A) showed a significant elevated allelic loss variant frequency in WS patients compared with age-matched individuals [34]. This genomic instability was also significantly higher in heterozygote carriers compared with wild-type siblings. Consistent with all these observations, several in vitro experiments have pointed to illegitimate recombination as being affected in WS cells. For example, elevated recombination was detected between small regions of homology within different parts of an introduced plasmid in WS cells when compared with wild-type cells [35]. Further experiments, on in vitro ligation reaction of plasmid DNA ends with WS cell extracts, have shown that deletions were due to recombination involving small regions of homology within the plasmid sequence [36]. Based on all these observations, WS is considered a genomic instability syndrome. Noticeably, genomic instability is an important hallmark of both cancer and the aging process. It will induce a number of changes at the DNA level, such as point mutations, deletions, hypermutability, chromosomal abnormalities or even degradation of DNA. Thorough analyses of immortalized WS hTERT-expressing cells are required to assess the involvement of genomic instability and telomere shortening in the process of aging.

WS gene product and DNA damaging agents

DNA damage is well known to be mutagenic in cells, and many chemicals can lead to DNA damage. There are several different repair mechanisms to cope with DNA damage in mammalian cells, and they may include (i) one-step reactions, (ii) single- and multistep base excision mechanisms, and (iii) multistep reactions involving several specific protein complexes. Many proteins involved in these mechanisms are exonucleases or helicases [reviewed in 37]. Any defect in DNA repair may lead to DNA lesions and eventually to mutations in important genes involved in cell cycle regulation or different aspects of cellular metabolism. Thus, these mutations may lead to either proliferative decline or neoplastic transformation. It is known that DNA damage can also lead to DNA breaks, which in turn can lead to illegitimate recombination and chromosomal abnormality [38]. Illegitimate recombination is known to be an important competing pathway with homologous recombination for chromosomal double-strand break repair in mammalian cells [39]. There is no significant increase in sister chromatid exchange in WS compared with normal cells, indicating that the overall rate of recombination is not increased in WS cells. Instead, the data suggest that the frequency of illegitimate recombination is higher in WS cells. For this reason, it has been postulated that the WS gene product may be a suppressor of illegitimate recombination or may affect some type of DNA recombinational repair.

Although DNA abnormalities have been detected in WS cells, these cells are not hypersensitive to a variety of chemicals or physical mutagens [3]. Moreover, no consistent DNA repair deficit has been identified in WS primary cells [40–42]. WS cells have been shown to be sensitive only to two classes of DNA-damaging agent: toposiomerase inhibitors and the chemical 4-nitroquinoline 1-oxide.

WS cells are sensitive to both the topoisomerase I inhibitor camptothecin and the topoisomerase II inhibitor etoposide [43–45]. Type I topoisomerase (which nicks DNA, creating a single-strand break) is involved in some aspects of replication and transcription. It has been identified as a cofactor of activator-dependent transcription by RNA polymerase II [46]. In the fly, topoisomerase I is also enriched in actively transcribed regions of the genome, including the nucleolus, where transcription of ribosomal RNA (rRNA) by RNA polymerase I occurs [47]. Mammalian type II topoisomerase (which cleaves both strands of DNA) [48] is involved in the terminal stages of DNA replication. It is also an important structural component of the mitotic chromosomal scaffold [reviewed in 49]. Camptothecin and etoposide stabilize the DNA/topoisomerase cleavage complex, resulting in DNA strand breaks that may not be re-ligated or repaired.

The 4-nitroquinoline 1-oxide agent is a procarcinogen that can be modified by specific cellular enzymes. Once modified into carcinogenic esters, these esters will alkylate guanine and adenine bases that eventually may lead to mutations if left unrepaired. The 4-nitroquinoline 1-oxide chemical can also induce an oxidative stress to cells [50]. It was found that WS cells are more sensitive to this procarcinogen than wild-type cells. Furthermore, this genotoxin differentiates heterozygotic carriers for WS mutations from wild type and homozygous mutant [19].

Like 4-nitroquinoline 1-oxide, γ-irradiation also induces oxidative stresses in addition to double-strand breaks. However, although γ-ray induces an oxidative stress, WS cells are not hypersensitive to γ irradiation. These results, along with the topoisomerase sensitivity data, indicate that the WRN locus is likely to be involved in the selective resolution of unusual DNA structures that arise only under certain conditions in the cell. These unusual DNA structures may also appear during DNA replication or transcription. In this respect, it is interesting to note that

in WS fibroblasts the S-phase and the whole cell cycle are prolonged [51]. An impaired S-phase transit was also detected in WS lymphoblastoid cell lines [52]. At the molecular level the rate of initiation of DNA replication is retarded in WS cells compared with control cells [53, 54]. Recent results have indicated that transcription efficiency of mRNAs in WS cells is reduced compared with cells from normal individuals as well [55]. Thus, as with topoisomerase I, these results implicate the WS gene product not only in some aspects of DNA replication but also in transcription.

The WS gene product contains a helicase and an exonuclease

The amino acid sequence of the human WRN gene product revealed two potential catalytic domains. One of them is a helicase domain, as it contains seven consensus motifs characterizing a subfamily of helicases containing a DEXH box sequence [8]. This subfamily includes the RecQ helicase prototype in *Escherichia coli* [9] as well as RecQL, RecQ4, RecQ5 and BLM helicases in humans [56–59]. It also includes Sgs1 from the budding yeast *Saccharomyces cerevisiae* and Rqh1 in the fission yeast *Schizosaccharomyces pombe* [60–62]. RecQ in *E. coli*, Sgs1 in *S. cerevisiae* and Rqh1 in *S. pombe* are considered orthologues of the human WRN gene rather than homologues. Homology at the amino acid level between these proteins and the WS peptide is high in the helicase domain but poor outside of it. In contrast, the mouse Wrn peptide and the *Xenopus laevis* FFA-1 gene product are true homologues of the human WRN gene, as the homology covers the entire sequence [63–65].
Interestingly, mutations in other RecQ helicase family members, like BLM and RecQ4 genes, lead to Bloom and Rothmund-Thomson syndromes, respectively [58, 59]. Although phenotypically Werner, Bloom and Rothmund-Thomson syndromes are different, they are all characterized by genomic instability. The phenotypic differences that we see between Werner, Bloom and Rothmund-Thomson syndromes is probably due to amino acid sequences outside the helicase domain of WRN, BLM and RecQ4 peptides. Such sequences presumably determine three-dimensional properties of the protein, the type of nucleic acid structures that can be recognized by the helicase domain, and the cellular proteins that can interact and differentially regulate each helicase [66]. In addition, one important difference between WRN peptide and the other members of the RecQ-like family is the presence of a potential exonuclease catalytic domain at its N-terminus portion, as revealed by advanced alignment analysis [67, 68].

Characteristics of the helicase domain

Several laboratories have demonstrated that the human WRN peptide has a 3′–5′ helicase activity in vitro, like RecQ in *E. coli* or Sgs1 in yeast [69–71]. It unwinds DNA-DNA duplexes as well as RNA-DNA heteroduplexes. This unwinding requires ATP, as a point mutation in the ATPase domain (or Walker A motif) abrogates the helicase activity [71]. WRN has a higher affinity for forked DNA structures and 3′-overhang double-stranded DNA [70]. However, it hardly unwinds blunt-end double-stranded DNA, and it does not have a higher affinity for DNA damage induced by the chemical 4-nitroquinoline 1-oxide [72]. Finally, the WRN peptide can unwind G2 tetrahelical structures of a d(CGG)$_n$ repeat sequences but is unable to unwind bimolecular tetraplex structures of a telomeric sequence [73]. This is in contrast to the Bloom helicase, another RecQ-like helicase which can efficiently unwind G4 DNA structures formed in vitro by G-rich sequences of telomeric repeats [74]. However, recent results have indicated that telomeric repeat DNA can form large noncovalent complexes with unique cohesive properties which are dissociated by the WRN helicase [75]. Such telomeric structures have been identified as extrachromosomal telomere repeat DNA in telomerase-negative immortalized cell lines [76]. Thus, the potential to specifically unwind unusual substrates may reflect the specific role that the WRN protein may have in the cell [73].

Characteristics of the exonuclease domain

In addition to its helicase activity, the WRN protein possesses an exonuclease activity within its N-terminus portion [77–79]. To date, it is the only RecQ-like helicase known to have an exonuclease domain. The exonucleolytic digestion of DNA remains functional in WRN peptide with an engineered mutation inactivating its helicase domain [71]. Moreover, a WRN peptide containing only the first N-terminal 333 residues retains the exonuclease activity [77]. Further characterization of the exonuclease domain indicated that the human WRN and mouse Wrn efficiently degrades the 3′ recessed strands of double-stranded DNA or a DNA-RNA heteroduplex. It has little or no activity on blunt-ended DNA duplex, DNA duplex with a 3′ protruding strand or single-stranded DNA. The WRN exonuclease can efficiently remove a mismatched nucleotide at a 3′ recessed terminus, and is capable of initiating DNA degradation from a 12-nt gap, or a nick [80]. A deletion mutant lacking the first 231 residues does not have exonuclease activity but still retains ATPase and helicase activity [79]. Likewise, point mutations in two important residues of the exonuclease domain (aspartate 82 and/or glutamate 84) abrogate its activity but do not affect the helicase domain [80]. Finally,

there is one report which suggests that the WRN peptide contains a 5′–3′ exonuclease activity as well [79]. This activity is absent in a WRN peptide lacking the first N-terminus 231 amino acids. However, other laboratories have failed to detect this activity in highly purified WRN peptide [77, 78].

Proteins known to interact with the WS gene product

Topoisomerases

The exact function of the WRN protein in cells remains elusive. A lot of effort has thus been invested in identifying cellular proteins that interact with WRN in order to shed some light on its possible functions. The first clue of a possible function of WRN in cells came with the topoisomerase I inhibitor experiments. The sensitivity of the WS cells to topoisomerase inhibitors suggested that there is a biochemical interaction between the WS peptide and one (or more) of the topoisomerases. Such biochemical interactions were already known to occur between helicases and topoisomerases in *E. coli* and *S. cerevisiae* species [60, 61, 81]. Consistent with these findings, an interaction between the WRN protein and topoisomerase I was recently detected in a human cancer cell line by coimmunoprecipitation studies [82].

SUMO-1

Topoisomerase-mediated damage (in the presence of topoisomerase inhibitors) represents a unique type of DNA damage. Abortion of the catalytic cycles of topoisomerases may result in cleavage complexes, or topoisomerase poisons, which in turn can lead to DNA breaks in the context of DNA replication or transcription. Recent studies have demonstrated that a small ubiquitin-like modifier protein (SUMO-1) can covalently bind to topoisomerase I or II cleavage complexes [83, 84]. SUMO-1 is believed to either activate a ubiquitin-like/ proteasome pathway, which degrades topoisomerase poisons, or to relocate the conjugated proteins, to a different site in the nucleus [83 and references therein]. Interestingly, SUMO-1 can also be covalently linked to the WRN protein [85]. This modification occurs between the exonuclease and the helicase domains of the WRN peptide. This kind of modification is likely to regulate nuclear WRN activity. It is unknown, however, whether this modification occurs only with topoisomerase-mediated damage or under other stressful cellular conditions.

WS gene product and the DNA replication complex

Since topoisomerases I and II are involved in DNA replication (and WS cells are sensitive to topoisomerase inhibitors), it is possible that the WS helicase interacts with these enzymes as a part of a replication structure. Indeed, we have seen that the mouse Wrn protein, but not a mutant protein, copurifies with the murine 17S multiprotein DNA replication complex through a number of purification steps [64, 82]. In addition, the WRN protein has been shown in vitro and in vivo to interact with two major components of the DNA replication complex, replication protein A (RPA) [86, 87] and proliferation cellular nuclear antigen (PCNA) [80, 82]. More important, the WRN protein has been shown to functionally interact with one subunit of the DNA polymerase *δ*. Finally, the p53 protein, which inhibits the DNA replication factor RPA in cells [88], can also interact with the WRN protein.

RPA

RPA associates with two other proteins to form a heterotrimer that acts as a single-strand binding protein. It is believed to stabilize displaced single-stranded DNA and to prevent reannealing during replication, transcription or DNA repair. It has been shown to facilitate the helicase activity of the WRN protein [71, 87].

PCNA

The interaction of the WS protein with PCNA requires a relatively short stretch of amino acids (168–246) in the N-terminal portion of the WS protein. These amino acids form part of the exonuclease domain and contain a D**Q**W**KL**LRD**FDV**K motif (important residues in bold). This motif is known to be critical for the interaction of several proteins of the replication complex with the so-called interconnecting loop domain of PCNA [89, 90]. It was recently determined that the WRN protein forms a homotrimer in vitro [80]. Interestingly, PCNA forms a homotrimer in the cells. Such data raise the possibility that the PCNA homotrimer increases the activity of the exonuclease domains of the WRN homotrimer toward specific substrates. More analyses are required to examine this point thoroughly. In any event, the interaction between PCNA and the WS protein is particularly interesting because PCNA is a potential communication point between a variety of important cellular processes, including cell cycle control, DNA replication, DNA recombination and DNA repair [reviewed in 90].

DNA polymerase *δ*

The best evidence for a specific function of WRN at the DNA replication/repair complex comes from data demonstrating the functional interaction between the WRN protein and yeast DNA polymerase *δ* [91]. WRN protein stimulates the rate of reinitiation of DNA synthesis of eukaryotic DNA polymerase *δ*. It does not affect

the activity of any other DNA polymerases. These results are consistent with earlier findings indicating that the homologue of WRN (FFA-1) is required for replication foci formation in *Xenopus* egg extracts [65]. Thus, the absence of WRN in a multiprotein DNA replication complex may contribute to the S-phase defects observed in WS cells.

p53

Another protein that, when mutated, can increase genomic instability is p53. Changes in the activity of p53 have been implicated in the onset of replicative senescence and apoptosis [reviewed in 92 and 93]. Two laboratories have demonstrated that the C-terminus portion of p53 binds to a C-terminus portion of the WRN peptide [94, 95]. It has been shown that WS fibroblasts have an attenuated p53-mediated apoptotic response, and this deficiency can be rescued by expression of wild-type WRN peptide [95]. An attenuation of a p53-apoptotic response in already genetically unstable WS cells would have implications for cancer progression. Consistent with this hypothesis, p53-null/Wrn-null mice show an increased mortality rate compared with p53-null or Wrn-null mice alone [96]. Interestingly, promoter analysis of the WRN gene has identified elements that can be controlled by the state of the retinoblastoma (Rb) and p53 gene products [97]. Rb increases the activity of the promoter of the WRN gene, whereas p53 downregulates it. These observations indicate a feedback mechanism by p53 on WRN expression that may affect apoptotic response.

WS gene product and transcription

Immunofluorescence studies with antibodies against the human WRN peptide have shown that WRN protein is densely localized to transcriptionally active nucleoli of cycling cells [98, 99]. In contrast to human cells, the mouse Wrn protein is not concentrated in the nucleoli but is found homogeneously in both the nucleoplasm and the nucleoli [14, 98, 99]. Interestingly, when the human WRN protein is expressed in mouse cells, the immunofluorescence staining pattern with anti-human WRN is nucleolar [100]. This distinction between mouse and human is probably due to the 30% difference in amino acid sequences seen in both species of WS gene products. Remarkably, a dramatic reduction of the WRN nucleolar signal concomitantly with a decrease in ribosomal DNA (rDNA) transcription was observed in quiescent human cells or in cells treated with 4-nitroquinoline 1-oxide [98]. These results point to a role of WRN protein in rDNA transcription.

The exact role of WRN protein in rDNA transcription is unclear, as there is no difference in the rate of transcription from a rDNA gene template between extracts from normal or WS fibroblasts [101]. In contrast, similar experiments have shown a 50–60% reduction in RNA polymerase II transcription of WS extracts compared with normal cell extracts [101]. This reduction in transcription would certainly affect the replicative growth of the WS cell. Further, experiments showed that there is a 27-residue motif repeated twice in the human WRN protein (but not in the mouse protein) which can promote transcription in a yeast system. Finally, evidence indicated that the WRN protein elevates the transcriptional activity of RNA polymerase II in vitro and in vivo [101, 102]. In fact, many laboratories have observed differential expression of several genes in WS cells compared with normal cells [102–104]. However, it is unknown whether the observed differential expression is due to a defect in the WRN activity itself during transcription, or a consequence of the accumulation of mutation in the genome of the WS cells. With the accession of DNA microarray technology, it will be possible to see whether only a specific subset of genes are regulated at the transcriptional level by the WRN protein. In any event, the WRN gene product points to a multifunctional protein that can affect DNA replication and transcription as well.

Function of RecQ-like helicases in lower organisms

The potential molecular function of the WRN protein has been greatly assisted by the analysis of RecQ-like protein in lower organisms. In particular, RecQ in *E. coli* is considered a suppressor of illegitimate recombination [105]. Most λ bacteriophages integrate by recombination at several DNA hot spots in the bacterial genome. These recombination events are increased in RecQ mutants. Furthermore, sequence analysis of recombination junctions in the transducing phages indicates that recombination takes place between short homologous sequences [105]. It was also found that RecQ mutations increase spontaneous ultraviolet (UV) light-induced illegitimate recombination at DNA replication forks [reviewed in 106]. Therefore, RecQ would be an important enzyme for dealing with structures generated at stalled or collapsed replication forks. Finally, RecQ helicase unwinds covalently closed double-stranded DNA substrates, and this activity specifically stimulates *E. coli* topoisomerase III to fully catenate double-stranded DNA molecules [81]. The activity of the helicase/topoisomerase complex would thus control DNA recombination. As some DNA replication defects during S phase have been detected in WRN mutations, it is possible that the WRN protein has a similar function in eukaryotes.

Another unicellular organism that is very useful in deciphering the potential function of RecQ-like enzyme is the budding yeast. Sgs1 in *S. cerevisiae* is the homologue of RecQ in *E. coli,* and it was identified by a search for

extragenic suppressors of the slow-growth phenotype in cells deficient for DNA topoisomerase III [60]. Cells with a mutation in topoisomerase III show a hyperrecombination phenotype that is also seen in cells with a mutation in Sgs1 [107]. Several laboratories have shown that Sgs1 is a 3′–5′ helicase which can interact with type I and II topoisomerases (topoisomerase III and topoisomerase I, respectively) [60, 61, 108]. Interestingly, Sgs1 mutants have a shorter life span than wild-type cells and phenotypically resemble aging yeast cells. This acceleration of aging is accompanied by recombination within the rDNA genes, leading to the accumulation of extrachromosomal rDNA circles and the fragmentation of the nucleoli [109, 110]. Using the appropriate expression vector, it was found that both the WRN and BLM genes suppress hyperrecombination in yeast Sgs1 mutant [111]. However, only the human BLM, but not WRN, can prevent premature aging in Sgs1 mutants. In addition, WRN does not prevent the increased homologous recombination at the rDNA loci caused by the sgs1 mutation [112]. As no extrachromosomal rDNA circle has been found in human WS cells, the exact relation between aging and extrachromosomal rDNA structures is still unclear. Nevertheless, the phenotype of mutations in the yeast helicase parallels the genomic instability found in WS cells [60, 61]. More important, the results showing the suppression of hyperrecombination in yeast Sgs1 by WRN protein do implicate the WRN gene in genomic stability. Interestingly, recent data have shown that Sgs1 deletion mutants are deficient in DNA repair and defective for induced recombination events that involve homologous chromosomes [113].

Possible functions of the WS gene product in mammalian cells

Based on all the observations mentioned in this review, it is tempting to speculate and say that WS may be a consequence of a defect in the resolution of recombination intermediates formed during replication or transcription. Processes such as DNA replication and transcription generate regions of single-stranded DNA which may inadvertently provide a substrate for the initiation of recombination. Various mechanisms have evolved to ensure that recombination does not occur promiscuously during these events. The WRN protein may be part of one such mechanism. Indeed, deletions and translocations are detected in WRN-deficient cells, pointing to a DNA recombination defect. These mutations can potentially inactivate tumor suppressor genes or activate oncogenes, accelerating tumor formation and/or progression.

The results reviewed here indicate that the WRN protein interacts with part of the multiprotein DNA replication complex. Several proteins known to be part of this complex can physically interact with WRN and include RPA, PCNA, topoisomerase I and DNA polymerase δ. It is important to emphasize that all these proteins are also involved in DNA repair. The topoisomerase I interaction is interesting, as most RecQ-like helicases studied have been demonstrated to interact with type I or II topoisomerases [60, 79, 112–116]. The specific sensitivity of WS cells to topoisomerase I inhibitors indicates that both helicase and topoisomerase enzymes are acting in concert at the replication fork. Topoisomerase I acts ahead of converging replication forks to release any stress on the replicated DNA. In the process of nicking the DNA, and before the religation step, the cleavage complex may by accident ‘freeze’ momentarily, creating a double-strand break as the replication complex runs into this topoisomerase/DNA cleavage complex. The drug camptothecin, used in several studies, recreates this incident at a very high frequency in cells. Another possibility is that unrepaired lesions ahead of the replication fork can stall the DNA replication complex or cause it to collapse. A recombination process must then occur to repair the double-strand breaks and to permit the replication complex to resume DNA synthesis. During this process, a single-stranded DNA can invade the other parental strand or the other sister chromatid to produce recombination and repair of the break. In this process, the single-stranded DNA may inadvertently hybridize to a short stretch of homology either further downstream of the replication fork, or to a wrong site on the sister chromatid, or to a short sequence on another chromosomal replicon close by in the nucleus. In such cases, the recombinational intermediate to be repaired may be recognized as ‘abnormal’ by the replication complex. The WRN protein would remove the invading DNA strand on the illegitimate stretch of short homology, thus inhibiting illegitimate recombination at the replication fork. The helicase and the exonuclease domains would be required to remove the invading strand for subsequent completion of proper homologous recombination at the replication fork (see fig. 1 for model). Thus, one possible function of the WRN protein is to monitor recombinational repair of double-strand breaks at the site of DNA replication. The absence of functional WRN may lead to illegitimate recombination during the repair of the break, creating small deletions in a gene or variegated chromosomal translocations (fig. 1). It is interesting to note that recently it was demonstrated that the WRN protein promotes translocation of Holliday junctions in vitro and colocalizes with RPA upon replication arrest [117]. In addition, one report has indicated that the WRN protein coimmunoprecipitates with p50 and p125 subunits of the DNA polymerase δ complex [118]. Finally, Ku86/70 complex, which binds to the ends of the double-strand breaks, interacts and stimulates the exonuclease (but not the helicase) activity of the WRN protein [119], again implicating WRN in DNA repair.

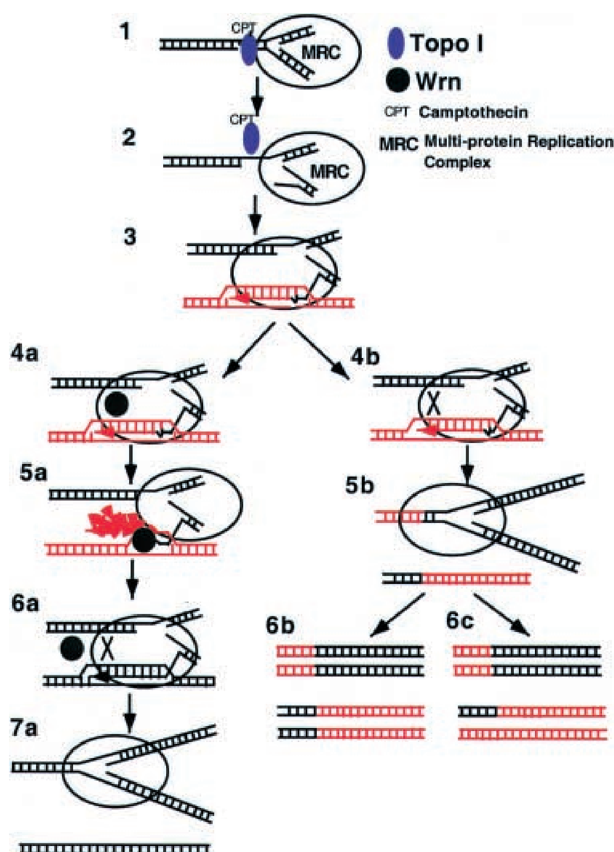

Figure 1. Model for the involvement of the WRN protein in suppression of illegitimate recombination at the DNA replication fork. Topoisomerase I enzymes (Topo I) work ahead of the replication fork to release any stress applied onto the replicated chromosome. The drug camptothecin (CPT) will inhibit the re-ligation step of the enzyme, leading to a double-strand break as the multi-protein DNA replication complex (MRC) runs into the cleavage complex (CPT-Topo I in 2). A single-stranded DNA may inadvertedly invade another strand from a small region of homology on another chromosome (in red) for a DNA repair recombination process (in 3). If the WRN protein (WRN) is present, this recombination intermediate may be recognized as being abnormal (by a yet unknown mechanism), and WRN protein may be called upon to remove the invading strand (in 4a and 5a). Subsequently, the single-stranded DNA may invade the right DNA template (in black) for proper homologous recombination and repair of the DNA at the replication fork (in 6a and 7a). If the WRN protein is mutated, illegitimate recombination may occur at a higher frequency (in 4b). This would lead to the synthesis of a mutated chromosome. Illegitimate recombination may lead to a deletion or translocation (5b and 6b). This type of recombination during replication may also lead to variegated chromosomal translocations (in 6c), which are often observed in WS cells.

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