Chapter 2
Role of RecQ Helicases in Nuclear DNA Repair and Telomere Maintenance

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Abstract Survival of an organism is reliant on preservation of genomic integrity. The RecQ helicase family of proteins plays crucial roles in maintaining genomic stability. DNA repair processes are very important for restoring the damaged DNA, and increasing lines of evidence suggest that RecQ helicases are involved in these processes. Telomeres are situated at the end of linear chromosomes, where they play key roles in the preservation of genome stability. Telomerase and telomere protein complexes play key roles in telomere length regulation. The latter, referred as the shelterin complex, also acts on telomere-specific structures and telomere capping. Other telomere-associated proteins are involved in the proper processing of telomere length, structure and capping. RecQ helicases, especially WRN, are also believed to be involved in the maintenance of telomeres. They are implicated in replication, recombination and proper repair of telomeric DNA.

Abbreviations

8-oxodG	8-oxo-7,8-dihydroguanine
ALT	Alternative lengthening of telomeres
BER	Base excision repair
BS	Bloom syndrome
DSB	Double-strand break
DSBR	Double-strand break repair
dsDNA	Double-stranded DNA
FISH	Fluorescence in situ hybridization
G4	G-quadruplex
HJ	Holiday junction
HR	Homologous recombination

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ICL	Interstrand crosslink
LP-BER	Long patch base excision repair
MMR	Mismatch repair
NER	Nucleotide excision repair
NHEJ	Nonhomologous end-joining
ROS	Reactive oxygen species
RTS	Rothmund–Thomson syndrome
RQS	RecQ Conserved
SBR	Single-strand break repair
SCE	Sister chromatid exchange
SP-BER	Short-patch base excision repair
ssDNA	Single-stranded DNA
TIF	Telomere dysfunction-induced foci
WS	Werner syndrome

2.1 Introduction

One group of proteins that is actively involved in maintaining genome stability is the RecQ helicase family, a highly conserved group of DNA helicases that function in the multiple DNA metabolic processes. There is only one RecQ homolog in *Escherichia coli*, RecQ, and one in each yeast, designated Sgs1 and Rqh1 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively. Curiously, five RecQ homologs have been identified in mammalian cells: RECQ1, BLM, WRN, RECQ4, and RECQ5. Three of the gene products have been shown to be associated with autosomal recessive disorders characterized by genomic instability and cancer predisposition. Bloom syndrome (BS), Werner syndrome (WS), and Rothmund–Thomson syndrome (RTS) are associated with defects in BLM, WRN, and RECQ4, respectively. The RecQ helicases have roles in DNA repair and replication, and they interact with proteins involved in telomeric maintenance. In this chapter we will discuss the roles of different RecQ helicases in DNA repair and telomeric DNA maintenance.

2.2 Telomere: Structure and Maintenance

The unidirectional nature of DNA synthesis prevents template-directed synthesis of the 5′-most ends of lagging strands during the DNA replication process. (Levy et al. 1992). This phenomenon is termed as “the end replication problem” (Watson 1972). Although prokaryotes use several different mechanisms to deal with this, eukaryotic cells have evolved a unique solution involving a special structure known as the telomere (Blackburn and Szostak 1984). Telomeres are situated at the ends of the linear eukaryotic chromosomes and consist of long stretches of short tandem DNA repeat sequences associated with specialized proteins. The unique structure

of the telomere plays an important role in the maintenance of telomeric DNA. In mammals, telomeres are composed of double-stranded tandem repeat sequences, followed by a single-stranded short 3′-overhang. Telomeres normally exist in a loop structure with the 3′-single-stranded overhang invading the telomeric dsDNA (Griffith et al. 1999). This so-called T-loop configuration is stabilized by telomere binding and associated proteins. Disruption of the T-loop and subsequent exposure of the 3′-overhang represent an uncapped state of telomeres. Figure 2.1 illustrates the uncapped and t-loop structures of the telomeric DNA and shows the proteins associated with telomere maintenance.

Telomeres prevent chromosome termini from being recognized as broken DNA ends (i.e., double-strand breaks; DSBs). Telomere dysfunction emanates from loss of telomere DNA repeats or loss of protection by telomere-associated proteins. Uncapped telomeres are subject to nucleolytic degradation and undesirable recombination mediated by homologous recombination (HR) or nonhomologous end-joining (NHEJ) processes. Uncapped telomeres are recognized by many DNA damage response proteins, including ATM, γ-H2AX, 53BP1, MDC1 and NBS1, form telomere dysfunction-induced foci (TIF), and can induce cell cycle arrest, senescence, or apoptosis (de Lange 2005; di Fagagna et al. 2003, 2004). Telomere attrition is frequently associated with aging (Harley et al. 1990) and premature aging syndromes (Opresko 2008). Several factors, including telomerase, the shelterin complex, and T-loop structure are critical in telomere maintenance.

The telomere nucleoprotein complex, known as the shelterin complex includes telomere-specific binding proteins and their associated proteins (de Lange 2002). In mammals, this complex includes proteins that bind to the dsDNA telomeric region, TRF1 and TRF2, a protein that binds to the ssDNA telomeric overhang, POT1, and their associated proteins TIN2 and TPP1. The telomere protein complex controls telomere length in *cis* by inhibiting the action of telomerase at the ends of individual telomeres.(Bianchi and Shore 2008) For example, overexpression of TRF1 and TRF2 causes telomere shortening, whereas a decrease of telomere-bound TRF1 promotes telomere lengthening in human cells (Smith and de Lange 2000;

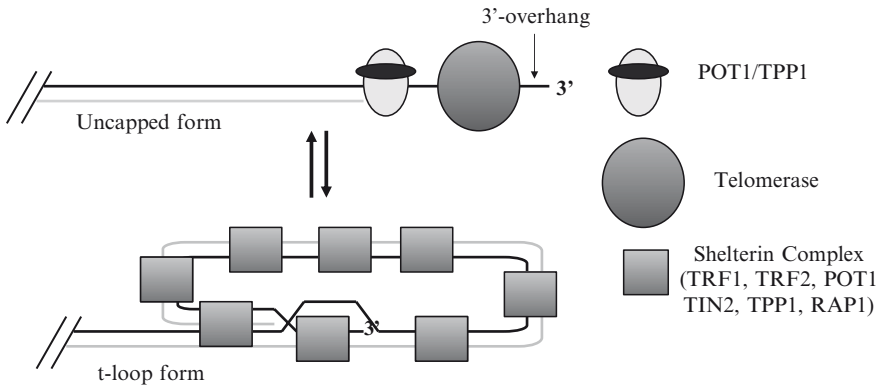

Fig. 2.1 Structure and protection of telomeric DNA

Van Steensel et al. 1998). POT1 and TPP1 form a heterodimer and modulate telomerase function by either negatively regulating telomerase access to the 3′-overhang, or serving as a telomerase processivity factor for telomere extension (Wang et al. 2007; Xin et al. 2007). The POT1/TPP1 heterodimer also binds along the length of telomeres via TRF1, which has been proposed to allow communication between the double-stranded telomeres and the recruitment of telomerase to the 3′-overhang. In fact, overexpression of a mutant form of POT1 lacking the DNA-binding domain abrogates TRF1-mediated telomere length regulation and induces a rapid and extensive telomere elongation (Loayza and de Lange 2003).

The telomere protein complex is also indispensable for telomere capping. Telomeres that are severely or completely stripped of the protective telomere protein complex trigger a DNA damage response (Karlseder et al. 2002; Hockemeyer et al. 2005; Wu et al. 2006). Mice deficient in shelterin proteins including TRF1, TRF2, POT1a (one of two mouse POT1 paralogs) or TIN2 die during the early embryonic development, demonstrating a vital role for the telomere protein complex in telomere capping (Karlseder et al. 2002; Hockemeyer et al. 2006; Chiang et al. 2004). Uncapped telomeres also become the substrates of HR or NHEJ repair. It has been shown that loss of TRF2 function in ERCC1/XPF or Ku70 deficient genetic backgrounds results in increased chromosome end-to-end fusions (Zhu et al. 2003), aberrant HR at T-loops or between the telomere sister chromatids (Celli et al. 2006). Similar telomere capping defects were observed in *Pot1* knockout mice (He et al. 2006).

Telomerase is another crucial component of telomeres that is responsible for the (actual) synthesis of new telomeric sequences. It is a large ribonucleoprotein complex that contains two core components: telomerase reverse transcriptase (Tert) and telomerase RNA (Terc). After DNA replication, telomerase is recruited to the 3′ telomeric overhang, which it extends using its integral telomerase RNA as a template. Telomerase activity is essential in preventing the replication-dependent telomere loss in highly proliferative cells and cancer cells. However, most human somatic cells possess low or undetectable telomerase activity. This results in replication-associated telomere shortening and consequently a progressive restriction of the replicative potential of somatic cells (Greider and Blackburn 1996). Mutations in the genes encoding telomerase core components are associated with several human genetic disorders, including dyskeratosis congenita (DKC), aplastic anemia, and pulmonary fibrosis. Patients with these diseases display an accelerated telomere shortening, which suggests that telomere length maintenance plays a role in the etiology of these disorders (Savage and Alter 2008). Early generation telomerase knockout mice do not show any obvious phenotypes (Blasco et al. 1997; Liu et al. 2000). However, after a few generations, these mice eventually exhaust their telomere reserves and their telomeres become short and dysfunctional (Blasco et al. 1997; Erdmann et al. 2004; Liu et al. 2000). It has been shown that such critically shortened telomeres trigger a DNA damage response, resulting in either apoptosis or cellular senescence mainly in highly proliferative tissues (Blasco 2007). As a result, late-generation telomerase knockout mice display pleiotropic phenotypes, such as infertility, shortened life span,

abnormal hematological profile, atrophy of the spleen and small intestine, and attenuated bone marrow stem cell proliferation (Blasco 2007).

2.3 RecQ Helicases in DNA Repair

Four major DNA repair pathways maintain the stability of the nuclear genome: (1) base excision repair (BER), which repairs oxidative DNA base modifications such as 8-oxo-7,8-dihydroguanine (8-oxodG), alkylation base damage and single-strand DNA (ssDNA) breaks; (2) nucleotide excision repair (NER), which repairs bulky helix-distorting DNA lesions; (3) double-strand break repair (DSBR); and (4) mismatch repair (MMR), which repairs single-nucleotide mismatches and small insertion–deletion mispairs. DNA repair pathways that act on the mitochondrial genome are less well characterized than the nuclear DNA repair pathways, but mitochondrial DNA repair is believed to have high biological importance. RecQ helicases play important roles in base excision repair and double-strand break repair. They are also implicated in resolving complex DNA structures like the G-quadruplex (Bohr 2008). The roles of RecQ helicases in various important genomic maintenance functions are illustrated in Fig. 2.2, and discussed in detail in this chapter.

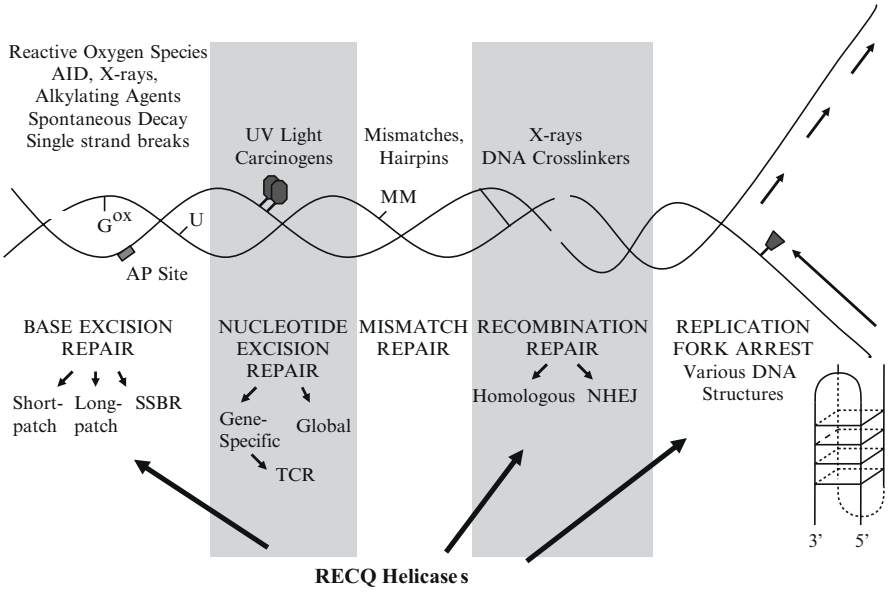

Fig. 2.2 DNA repair pathways and involvement of RecQ helicases

2.3.1 WRN and BLM in DNA Repair

2.3.1.1 Double-Strand Break Repair

DSBs are induced by ionizing radiation and other DNA-damaging agents. They are recognized by DNA damage-sensing proteins, leading to the formation of DNA repair foci enriched in phosphorylated histone H2AX (γ-H2AX). These foci can be detected by immunological or biochemical/cell biological methods. DSBs are repaired by homologous recombination (HR) or nonhomologous end-joining (NHEJ).

RecQ helicases appear to play major roles in DSBR. Rad51, which is a key player in the strand invasion event during HR, interacts with WRN (Otterlei et al. 2006) and BLM (Wu et al. 2001). The physical interaction between BLM and Rad51 stimulates branch migration by BLM on Holliday junctions. Rad52 both inhibits and enhances WRN helicase activity in a DNA structure-dependent manner, whereas WRN increases the efficiency of Rad52-mediated strand annealing (Baynton et al. 2003), suggesting that Rad52 and WRN may cooperatively facilitate the rescue of stalled or blocked DNA replication forks. Rad54, another key protein in this pathway, co-localizes with WRN in response to replicative stress (Otterlei et al. 2006). WRN also associates with the Mre11-Rad50-NBS1 complex via NBS1 (Cheng et al. 2004) and the tumor suppressor BRCA1 (Cheng et al. 2006). Some of these protein interactions are functional; for example, BRCA1 stimulates WRN helicase, which is required for HR in cell extracts. Furthermore, WS cells are deficient in the removal of DNA interstrand cross-links (ICL), a process that requires recombination (Cheng et al. 2008; Poot et al. 2001).

WRN and BLM both play roles in the assembly of DSBR complexes at γ-H2AX foci, an early step in DSBR. It was recently observed that WRN can act upstream of ATM after the exposure of cells to agents that cause replication fork collapse (Cheng et al. 2008). However, WRN and BLM may also play downstream roles in NHEJ and DSBR. For example, WRN interacts with the Ku70/80 heterodimer, a primary mediator of NHEJ (Karmakar et al. 2002b), and this interaction strongly stimulates WRN exonuclease activity in vitro (Cooper et al. 2000). WRN also interacts with the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) (Karmakar et al. 2002a), suggesting that WRN might participate in NHEJ and/or telomere repair. We recently observed that WRN functionally interacts with the ligase IV complex (Kusumoto et al. 2008). However, WS cells are not particularly sensitive to γ-irradiation, which generates DSBs. It remains possible that WRN participates in a NHEJ subpathway or in end-joining in a subgenomic region such as telomeres or rDNA.

2.3.1.2 Base Excision Repair

Oxidation of macromolecules, especially DNA, may play a major role in aging, cancer and neurodegeneration. Oxidative DNA base modifications are caused by

endogenous reactive oxygen species (ROS), which are normal byproducts of oxidative phosphorylation in mitochondria and other metabolic processes. It has been estimated that 50,000–100,000 oxidative DNA lesions are generated per mammalian genome per day (Lindahl 1993). If these lesions persist, they can inhibit DNA replication and transcription, increase the frequency of point mutations and chromosomal rearrangements, and induce cellular stress, apoptosis or cell cycle arrest.

Oxidative DNA base damage is removed by BER. BER involves five steps: removal of a damaged base by a DNA glycosylase, incision of the phosphodiester backbone by an apurinic/apyrimidinic (AP) endonuclease, diesterase- or lyase-mediated modification of the DNA termini, DNA synthesis to fill in gapped DNA, and ligation of nicked DNA by DNA ligase. BER proceeds either via single-nucleotide replacement (i.e., short-patch (SP-BER)) or multiple nucleotide strand displacement (i.e., long patch (LP-BER)). SP-BER usually involves DNA polymerase β (POLβ) while LP-BER typically utilizes the replicative, PCNA-dependent polymerases POLδ or POLε, although POLβ can also be involved. The LP-BER pathway also involves the flap endonuclease FEN1, which removes the protruding ssDNA flap generated by DNA strand displacement. As will be discussed below, some mammalian RecQ helicases interact with and modulate the activity of BER proteins.

E. coli RecQ is the prototypical member of the RecQ helicase family. The helicase domain is highly conserved among RecQ family members, and all RecQ homologs, are active as DNA helicases in vitro. The RQC (RECQ conserved) domain, also present in *E. coli* RecQ, is less well conserved than the helicase domain, but is present in most RecQ family members. WRN is unique in the RecQ helicase family in having an intrinsic 3′–5′exonuclease activity. WRN exonuclease degrades DNA substrates with a 5′-overhang, but WRN also degrades the blunt-ended DNA structures, bubble or forked DNA and mismatch-containing DNA (Newman et al. 2008; Sharma et al. 2006; Shen and Loeb 2000). WRN and BLM have intrinsic DNA-dependent ATPase activity and ssDNA annealing activity (Wu and Hickson 2006). Although the biological significance of the ssDNA annealing activity remains to be determined, it has been proposed that this activity may facilitate strand migration during recombination or replication fork regression at the site of DNA damage in vivo (Wu and Hickson 2006). The precise biological functions of these distinct domains are not yet fully understood. WRN and BLM possess 3′–5′ DNA helicase (DNA unwinding) activity. The preferred substrates of these enzymes resemble intermediates in HR such as Holliday junctions and G-quadruplex structures (Mohaghegh et al. 2001). WRN and BLM also have a high affinity for normal and blocked or collapsed replication forks and telomeric structures. The substrate specificity of WRN and BLM helicases is rather similar. Because WRN and BLM interact with each other physically and functionally (von Kobbe et al. 2002), they might function synergistically on some DNA substrates; an unresolved, but important question.

Many WRN protein–protein interactions are mediated by the RQC domain. This noncatalytic region also binds DNA (von Kobbe et al. 2003) and contains a nucleolar

targeting signal, which localizes WRN to nucleoli in unstressed cells (von and Bohr 2002). APE1, the endonuclease that incises abasic sites during BER, inhibits WRN; this interaction could possibly prevent promiscuous unwinding of DNA repair intermediates (Ahn et al. 2004). WRN and BLM also stimulate DNA polymerase β, enhancing base incorporation and facilitating DNA strand displacement (Harrigan et al. 2003, 2006). WRN and BLM strongly stimulate FEN1 (Brosh et al. 2002; Sharma et al. 2004). In vitro evidence also indicates that WRN exonuclease can act as an autonomous proofreading enzyme for DNA polymerase β during LP-BER (Harrigan et al. 2007). Collectively, these data indicate that WRN could participate in BER, specifically in LP-BER (Harrigan et al. 2006). Using an assay for LP-BER, we demonstrated that this process was defective in WS cells (Harrigan et al. 2006). Although WRN is not essential for BER, WRN-deficient cells accumulate 8-oxoG (Das et al. 2007) and are sensitive to some DNA-damaging agents that generate BER substrates (Blank et al. 2004; Harrigan et al. 2006). Although WRN does not appear to interact with human OGG1, the major glycosylase for 8-oxoG in human cells, it does interact in vivo and in vitro with NEIL1 (Das et al. 2007), a human glycosylase for formamido-pyrimidine (Fapy) (Das et al. 2007; Imoto et al. 2006). These lesions are common (Hu et al. 2005a), but not very well characterized (Jaruga et al. 2004), and they accumulate in cells deficient in WRN (Das et al. 2007). WRN also interacts functionally and reciprocally with polyADP ribose polymerase (PARP-1), a protein with a key role at various steps during BER/SSBR. PARP-1 ribosylates a large number of cellular proteins, but it does so at a lower level in WRN-deficient cells, suggesting that PARP-1 is activated or stimulated by WRN (von Kobbe et al. 2002). PARP-1 also co-localizes with RECQ4 (Dietschy et al. 2007). Thus, WRN plays several roles in BER/SSBR, but it is largely unknown whether other human RECQ helicases also participate in this process.

2.3.2 Other Helicases in DNA Repair

2.3.2.1 RECQ4

Rothmund–Thomson syndrome (RTS) is a rare, autosomal recessive disorder associated with a characteristic skin rash (poikiloderma) that begins in infancy, small stature, skeletal dysplasia, radial ray defect, sparse hair and eyebrows and occasional cataract formation. *RECQ4* mutations can cause RTS, RAPADILINO (Radial and patellar aplasia) and Baller-Gerold (bilateral radial aplasia and craniosynostosis) syndromes. Two-thirds of RTS patients have a mutation in *RECQ4*, but the cause of RTS in the remaining patients is not known (Wang et al. 2003). Approximately one-third of RTS patients develop osteosarcomas at a median age of 11.5 years, and all of these patients carry mutations in *RECQ4*.

The biological function of RECQ4 and the cellular pathways in which it is involved remain poorly understood. Some RTS cell lines have moderately increased sensitivity to dialkylating and other DNA-damaging agents. One RTS cell line is hypersenstivite to hydrogen peroxide. However, embryonic fibroblasts from RECQ4-deficient mice are not sensitive to UV or IR. In contrast to BS and WS cells, RTS cells are relatively resistant to the carcinogen 4-NQO (Jin et al. 2008). In addition, a recent study reported that RTS cells are more sensitive to DNA damage during S-phase (Jin et al. 2008).

Interestingly, RECQ4 exists in both the nuclear and cytoplasmic compartments of the cell (Yin et al. 2004). In the nucleus, RECQ4 is localized to promyelocytic leukemia (PML) bodies and DSB-induced Rad51 foci, sites of DSB repair activity. Cells from RTS patients have defects in sister chromatid cohesion, resulting in mosaic trisomies and isochromosomes. Thus, RECQ4 may participate in DSBR, sister chromatid exchange (SCE) and chromatid separation.

2.3.2.2 RECQ5

RECQ5 exists in at least three isoforms: RECQ5α (410 amino acid residues), RECQ5γ (435 residues) and RECQ5β (991 residues). All three isoforms include core helicase motifs. Defects in RECQ5 are not yet associated with any human disease. However, mutations in *RECQ5* may lead to chromosomal instability, cancer and premature aging, at least in model organisms. In *Caenorhabditis elegans*, deficiency in RECQ5 reduces life span (Jeong et al. 2003). *Recq5* knockout mice are phenotypically normal but cancer prone (Hu et al. 2007). Mouse embryonic stem cells that lack *Recq5* function have an elevated level of SCE, comparable to that caused by defects in *Blm*. Chicken *recql5* cells do not have high frequency SCE, but *recql5/blm* chicken and mouse cells have a higher frequency of SCE than *blm* cells. This suggests that RECQ5 suppresses SCE in chicken cells when BLM function is compromised.

RecQ helicases possess two domains which form the catalytic core of the enzyme, the DExH helicase and RECQ-Ct (RECQ C-terminal) domains. Some RecQ helicases also have a HRDC (Helicase and RNase D C-terminal) region; however, RECQ5β lacks this domain. The C-terminal portion of RECQ5β possesses an efficient DNA strand-annealing activity (Garcia et al. 2004). This domain is required for unwinding lagging-strand duplex DNA and for DNA strand exchange. Therefore, RECQ5 possesses a 3′–5′ DNA helicase activity, single-strand DNA-annealing activity, and can catalyze the branch migration of Holliday junctions. Although SCE is not elevated in RTS cells, inactivation of *Recq5* in mouse embryonic stem cells does increase the SCE frequency (Hu et al. 2005b). RECQ5β localizes to replication foci in cells exposed to hydroxyurea, UV and *cis*-platinum. RECQ5β interacts with PCNA in vitro and in vivo, Top3α, Top3β, Rad51 and RNA polymerase II. In addition, RPA stimulates the rate and extent of strand exchange by RECQ5β on a 3′ flap substrate (Garcia et al. 2004).

2.4 RecQ Helicases in Telomere Maintenance

Maintenance of telomeres is essential for the conservation of genomic integrity. As mentioned earlier, telomerase and the shelterin complex play key roles in telomere length maintenance. In addition, other proteins are also involved in the direct or indirect protection of telomeres. Some are directly involved in the telomere capping (briefly described before) while the others participate in several other processes leading to the proper preservation of telomeres.

A number of DNA repair and damage checkpoint proteins have been found to associate with telomeres including ATM, ATR, components of HR, NHEJ (KU/DNA-pkc) or DSBR (MRE11/NBS1/RAD50 complex), NER/BER proteins (ERCC1/XPF, PARP1/PARP2, FEN1), DNA helicases and nucleases (WRN, BLM, Apollo), and DNA topoisomerase. Many of these DNA repair/damage checkpoint proteins are actively involved in telomere length homeostasis, possibly by assisting in telomere DNA repair and telomere capping. RecQ helicases, especially WRN, are known to play a significant role in the proper maintenance of telomeres. A strong argument for the involvement of WRN in telomere processing is that *Wrn* and *Terc* double knockout mice have phenotypes resembling the clinical features of WRN deficient humans (Chang et al. 2004). In vivo studies have shown that the extent and rate of telomeric repair is lower in WS patients (Kruk et al. 1995). This notion is further supported by an accelerated telomere loss observed in WS cells (Crabbe et al. 2007).

Several studies have illustrated possible mechanisms by which WRN may contribute to the regulation of telomere length. A prominent role for WRN also has been suggested in telomeric DNA damage responses (Eller et al. 2006). WRN and BLM physically and/or functionally interact with the telomeric DNA protecting proteins POT1, TRF1 and TRF2 (Opresko et al. 2002, 2004, 2005) (Fig. 2.3). WRN also associates with Mre11, Rad50, Nbs1 and these proteins in turn interact with TRF2. WRN activity is affected by Ku and this protein interacts with TRF1 and TRF2. WRN helicase activity is also modulated by POT1. WRN acts efficiently on telomeric t-loop/D-loop structures but does not show any activity on non-telomeric

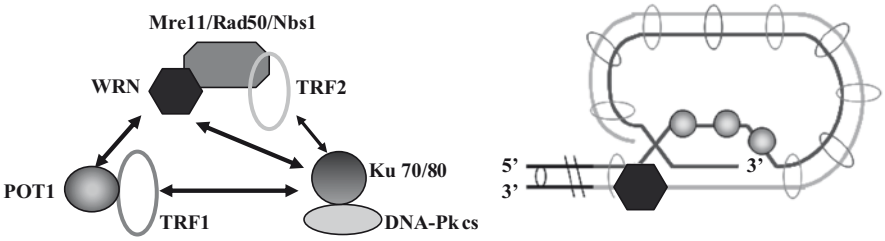

Fig. 2.3 WRN interacts with telomere binding and associated proteins abd telomeric t-loop DNA

DNA with the same structures. WRN's helicase activity on D-loops is also modulated by POT1. Figure 2.3 illustrates the interaction of WRN with the various proteins associated in telomere maintenance and telomeric t-loop DNA

The RecQ helicase activities involved in several important telomeric processes are illustrated in Fig. 2.4. WRN and BLM unwind the telomeric D-loop to initiate DNA replication. Telomeric DNA can form secondary structures involving guanines. For example, G-quadruplex (G4) structures are formed in telomeres. WRN and BLM also preferentially unwind the G4 structures and thus might be involved in resolving the G4 structures at telomeres. Telomeric DNA is also prone to numerous types of DNA damage including oxidative damage, double-strand breaks, and single-strand breaks. WRN is believed to play important roles in processing and repairing of damaged telomeric DNA. This helicase can take part in dissociation of complex structures as well as can interact with proteins involved in DNA repair.

2.4.1 Replication

WRN and other RecQ helicases have important functions in resolving potential impediments in telomeric DNA replication that can stall or block the replication forks.

Early evidence of involvement of WRN in replication comes from the fact that WS patients show extended S-phase. These patients are also very sensitive to agents that cause replication fork blocks (Opresko et al. 2003). Co-localization of WRN with RPA at nuclear foci was observed after hydroxyurea treatment. More importantly, WRN was also found at telomeres even in the absence of any

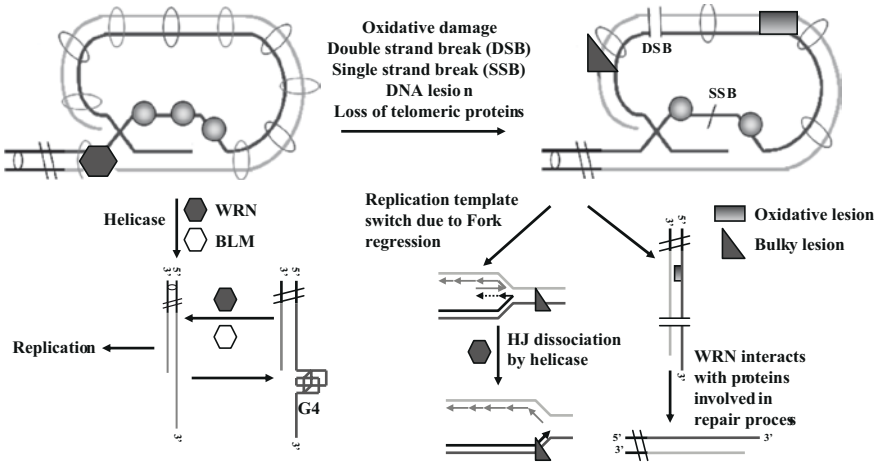

Fig. 2.4 Role of RecQ helicases in telomere maintenance and processing

replication blocking agents. This indicates an important role of WRN in telomeric DNA replication. However, experiments show that WRN is associated with only 5% of telomeric S-phase fibroblast suggesting that it might not participate in general replication of telomeres (Opresko 2008). It is more likely that WRN is recruited to the replicating telomeres in response to replication stress. CO-FISH studies by Crabbe et al. (2004) also suggest the requirement of WRN as part of an alternative mechanism to resolve relatively rare, but lethal events during telomere replication.

Replication fork termination in telomeres would be particularly damaging due to the difficulty in restarting replication in the distal portions of chromosomes. Replication of telomeric DNA requires the dissociation of the D-loop/t-loop structures. WRN and BLM unwind the D-loop structures to release the invading strand in vitro and the action of WRN is regulated by TRF1 and TRF2 (Opresko et al. 2004). TRF2 in particular interacts physically and enhances the helicase activity of WRN and BLM at telomeric D-loop structures (Opresko et al. 2002). The telomeric single-strand binding protein POT1 also improves the D-loop unwinding ability of WRN and BLM in vitro (Opresko et al. 2005). Restitution of stalled replication forks is an important mechanism to continue the replication process following any damage resulting lesion. This may include recombination, DNA synthesis or repair of the lesion, depending on the nature and position of the damage. RecQ helicases have a role in this process. WRN can function in homologous recombination of broken replication forks as well as in translesion DNA synthesis to bypass the lesion. WRN also unwinds model regression forks and enhances FEN-1 endonuclease activity to cleave the unwound 5′-strand in vitro, and WRN and FEN-1 co-localize at PCNA foci after the induction of stalled replication forks in vivo (Sharma et al. 2004).

Another potential block to the replication of the invading strand could be the formation G-quadruplex (G4) structures. In vitro studies confirmed the formation of these structures in telomeric $(TTAGGG)_n$ strands. Kamath-Loeb et al. (2001) reported that WRN can prevent stalling of replication at G4 DNA. Furthermore, bimolecular G4 structures are favored substrates for WRN and BLM. POT1 is also known to resolve the G4 structures and it interacts with WRN and BLM. These three proteins could thus work together to dissociate G-quadruplex structures in telomeres.

2.4.2 Recombination

Rare cells can survive the critical shortening of telomeres in the absence of telomerase activity by engaging a pathway known as ALT (alternative lengthening of telomeres).This pathway involves multiple telomere binding and recombination. In budding yeast, the RecQ helicase Sgs1 functions in a recombination-dependent ALT pathway. When critically short telomeres undergo recombination to try to restore the telomeric length, Sgs1 acts in resolution of these recombination inter-

mediates. It has been found that WRN and BLM can partially substitute for the function of Sgs1 in type II ALT (Cohen and Sinclair 2001; Mandell et al. 2005). A fraction of telomeric DNA from human ALT cell lines co-localizes with WRN and BLM (Opresko 2008). In vitro studies indicate that the recombination intermediates such as Holliday junctions and D-loops are excellent substrates for WRN and BLM. As discussed previously, these two RecQ helicases also function in resolving recombination intermediates generated during DNA repair. In addition to functioning in the DSB pathway, the Ku 70/80 heterodimer also suppresses the recombination at telomeres. As WRN interacts physically with Ku and POT1, it may function with Ku or POT1 in suppressing recombination intermediates.

2.4.3 Repair of Oxidative Damage

In vitro analysis has shown that telomeric DNA is prone to oxidative damage because of its G-rich content. Guanine has the lowest oxidation potential among the nucleobases and the GGG sequence found in telomeres has an even lower oxidation potential. Hence, telomeric DNA is susceptible to oxidative damage and can contain lesions like 8-oxoguanine (8-oxodG). Numerous studies have indicated an association between oxidative damage and telomere shortening (Newman et al. 2008; Satoh et al. 2008). Oxidative damage is repaired by the BER process. As mentioned earlier, WRN is believed to take part in BER and physically interacts with several proteins involved in BER. Oxidative damage can result in DSB in telomeres and WRN is also implicated in DSB repair processes. Recently, we have seen that WRN and BLM interact with the in vitro D-loop structures containing 8-oxodG lesions and they unwind these substrates more efficiently than the undamaged D-loops (unpublished results). However, a great deal of work needs to be done to assess the exact role of WRN in the repair of oxidative damage of telomeres. It is still fairly unclear whether and how BER operates at the telomere end.

2.5 Perspective

Our understanding and knowledge of the exact roles of RecQ helicases in DNA repair and telomere maintenance is still inadequate. Involvement of RecQ helicases in most of the important DNA repair and maintenance pathways is clear, but their exact functions and mechanisms are not obvious. Also, WRN and to some extent BLM and RecQ4 are the most studied RecQ helicases, while very little is known about the functions of other RecQ helicases in the maintenance of genomic stability and telomeres.

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