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RecQ helicases in DNA double strand break repair and telomere maintenance

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Abstract

Organisms are constantly exposed to various environmental insults which could adversely affect the stability of their genome. To protect their genomes against the harmful effect of these environmental insults, organisms have evolved highly diverse and efficient repair mechanisms. Defective DNA repair processes can lead to various kinds of chromosomal and developmental abnormalities. RecQ helicases are a family of evolutionarily conserved, DNA unwinding proteins which are actively engaged in various DNA metabolic processes, telomere maintenance and genome stability. Bacteria and lower eukaryotes, like yeast, have only one RecQ homolog, whereas higher eukaryotes including humans possess multiple RecQ helicases. These multiple RecQ helicases have redundant and/or non-redundant functions depending on the types of DNA damage and DNA repair pathways. Humans have five different RecQ helicases and defects in three of them cause autosomal recessive diseases leading to various kinds of cancer predisposition and/or aging phenotypes. Emerging evidence also suggests that the RecQ helicases have important roles in telomere maintenance. This review mainly focuses on recent knowledge about the roles of RecQ helicases in DNA double strand break repair and telomere maintenance which are important in preserving genome integrity.

Keywords

RecQ helicases; DNA double strand break repair; Werner syndrome; Bloom syndrome; Rothmund Thomson syndrome; telomere maintenance

Introduction

Living organisms encounter various kinds of environmental insults, both exogenous and/or endogenous, which can adversely influence the stability of their genomes. These environmental stresses introduce many abnormalities in the genome, ranging from base damages, replication blockage, DNA cross-links, telomeric defects to DNA double strand breaks (Fig. 1). To counteract and protect their genomes against the harmful effects of these environmental exposures, organisms have evolved highly efficient DNA repair mechanisms. Defects in these diverse repair pathways have deleterious consequences to the cell such as

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chromosomal or developmental defects, or various kinds of cancers/aging phenotypes. One such group of repair proteins that are actively engaged in various aspects of DNA metabolism is the RecQ helicases. The RecQ helicases are evolutionarily conserved, DNA unwinding proteins, which help in the maintenance of genome integrity by participating in many DNA metabolic processes, DNA repair pathways and transcription which are summarized in Fig. 1 [1–3].

The RecQ helicase are conserved from prokaryotes to higher eukaryotes. Lower organisms such as bacteria and yeast possess only one RecQ homolog, whereas higher eukaryotes including mammals possess multiple forms of RecQ helicases. These multiple RecQ helicases either possesses unique and/or overlapping functions depending on the types of DNA damage and repair pathways. Therefore, it is likely that during the evolutionary process the functions of the RecQ helicases have diversified to adapt to the changing environment and complexity of genomes. The hallmark feature of all the members of the RecQ helicase family is the conserved helicase domain which is crucial for their functions. Five RecQ homologs have been found in humans and mice namely, RECQL1, BLM, WRN, RECQL4 and RECQL5. Defects in three of these have been associated with rare genetic disorders characterized by genome instability, multiple cancer predispositions and/or a premature aging phenotype. Werner syndrome (WS) is caused by defects in WRN (except in a few cases when clinically indistinguishable WS is caused by defects in lamins), Bloom syndrome (BS) is due to defects in BLM, and Rothmund Thomson (RTS), RAPADILINO and Baller Gerold (BGS) syndromes are associated with defects in RECQL4. The other two members, RECQL1 and RECQL5, have not yet been linked to any disease phenotype, but studies in humans and mice have suggested their important roles in genome stability [4].

RecQ helicases are involved in base excision repair (BER), DNA double strand break repair (DSBR), intra-strand cross link repair (ICL), recovery of stalled replication forks, and telomere processivity and stability (Fig. 1) [3–6]. One finding suggests the involvement of RECQL4 in NER pathway by its interaction with XPA, a key protein involved in NER pathway [6]. However, the involvement of RecQ helicases in NER is still obscure. Recent studies in *Xenopus* as well as in humans also indicated that one of the RecQ helicase members, RECQL4, is an important component of the DNA replication machinery and is a part of the DNA replication initiation complex [7–9]. Another RecQ helicase, RECQL5, interacts with RNA pol II, suggesting its involvement in transcription [10, 11]. Therefore, RecQ helicases play diversified roles in genome stability and have been called the “guardians of the genome”. This review mainly focuses on important functions of RecQ helicases in DNA double strand break (DSB) repair and telomere processing which are crucial for maintaining genome stability.

1. DNA double strand break repair

DSBs are very potent and deleterious forms of DNA damage in the genome, and if left unrepaired they can cause cell cycle arrest, mutagenesis, gross chromosomal rearrangements, cell death and tumorigenesis. DSBs can arise spontaneously during normal DNA metabolism or when cells are exposed to DNA damaging agents or ionizing radiations. In higher eukaryotes, DSBs are mostly repaired by two distinct pathways i.e., homologous recombination (HR) and non-homologous end joining (NHEJ) [12]. The different steps of both of these pathways and proteins that interact with RecQ helicases are summarized in Fig. 2. The HR pathway is preferential in the late S-G2 phase, whereas NHEJ mainly plays a dominant role in the G1 to early S-phase of the cell cycle [13]. The HR pathway is a high fidelity repair mechanism which requires homologous sequences primarily from the sister chromatids. In contrast, the NHEJ pathway is an error prone mechanism involving the

joining of two ends of a DSB via a process that is largely independent of terminal DNA sequence homology [14–17].

1.1 RecQ helicases in DSB repair

RecQ helicases are actively involved in DSB repair. Some of the RecQ helicases are recruited at an early stage to the site of DSBs [18–21]. They interact with key DSB repair proteins at multiple stages of both the HR and the NHEJ pathways of DSB repair, and modulate their functions.

When a DSB is detected, a complex network of signaling proteins involved in DSB repair get activated leading to extensive chromatin restructuring at and/or around the DSB. The DSB ends are first recognized by the Mre11-Rad50-Xrs2 complex in budding yeast or the MRE11-RAD50-NBS1 complex in multicellular eukaryotes (Fig. 2) [22]. The DNA ends are then resected in a 5′-3′ direction by the endo/exonuclease activity of Mre11 in complex with Sae2 endonuclease to generate free 3′ ssDNA termini. The DNA ends are further extensively resected either by Exo1 or the Sgs1-Dna2 pathway. At this initial step of end resection, RecQ helicases are actively involved (Fig. 2). The protruding 3′ ssDNA overhang is coated by RPA, after which Rad51 is recruited and displaces RPA from the ssDNA leading to the formation of Rad51 nucleoprotein filaments [23, 24]. The Rad51 nucleoprotein filament then catalyzes the ssDNA strand exchange reaction with the identical strand in the homologous duplex of the genome through complementary base pairing resulting in the formation of a displacement loop (D-loop). The D-loop facilitates the repair synthesis using the intact homologous sequence as the template strand and invading ssDNA as a primer for DNA polymerase during DNA repair synthesis. At this stage, RecQ helicases function in disrupting the Rad51 nucleoprotein filaments or preventing D-loop formation to prevent illegitimate recombination events. Further, the D-loop is resolved by branch migration activity by two different pathways: (a) synthesis dependent strand annealing (SDSA) in which the DNA strand reanneals to the original template or (b) by the formation of a double Holliday Junction (DHJ) which can be resolved by Sgs1 or the BLM complex. Therefore, members of RECQ helicases perform distinct functions at various steps during the HR pathway: they help in the initial step of end resection, disrupt Rad51 filament formation, and resolve DHJs by branch migration activity.

Another major pathway of DSB repair is NHEJ (Fig. 2). In the initial step of this repair process, a ring shaped Ku heterodimer (Ku70/80) binds to both ends of the broken DNA molecule [25, 26]. Then Ku recruits DNA dependent protein kinase (DNA-PK_{CS}), a serine/threonine protein kinase that is activated by DNA damage [27]. The final step in the repair process involves the assembly of the XRCC4/ligase IV complex at the DNA ends which is required for the ligation of the two cohesive DNA ends. The RecQ helicase member, WRN, forms a trimeric complex with both Ku70/80 and DNA-PK_{CS} as well as with the XRCC4/ligase IV complex and modulates their functions [28–30]. The involvement of RecQ helicases at different stages of both HR and NHEJ pathways are summarized in Fig. 2.

1.1.1 WRN in DSB repair—Evidence suggests that WRN is actively involved in the HR pathway of DSB repair. The WRN protein helps to resolve the RAD51-mediated HR intermediates in the cell. The Werner syndrome (WS) phenotype includes defective recombination resolution, mitotic arrest, cell death, or genomic instability [31].

DSBs can also be formed during the normal DNA metabolic processes, i.e., at stalled replication forks. DNA interstrand cross-links (ICLs) cause replication forks to stall, eventually leading to generation of one-sided DSBs near the ICL site. Since cells lacking WRN are hypersensitive to ICLs, WRN is likely involved in the repair of ICLs and the restoration of normal replication forks in the cell [32]. Consistent with this hypothesis, WRN

relocates to the sites of arrested replication induced by ICLs, where it physically and functionally interacts with RAD52 [33]. Cheng et al. showed that WRN cooperates physically and functionally with BRCA1 in cellular response to ICLs. The BRCA1/BARD1 complex associates with WRN and stimulates WRN helicase activity on forked and Holliday junction (HJ) substrates which is required to process the DNA containing ICLs [34]. Moreover, WRN also cooperates with the Mre11 complex both *in vivo* and *in vitro* via its association with Nbs1 [35]. Otterlei et al. also showed that WRN participates in a multiprotein complex containing RAD51, RAD54, RAD54B and ATR in cells where replication has been arrested by ICLs. These findings suggest that WRN plays a significant role in the recombination step of ICL repair [36].

Although the trigger(s) recruiting WRN to stalled replication sites are largely unknown, available evidence suggests that the involvement of WRN in response to replication stress is ATM/ATR-dependent [37]. In addition, several lines of evidence support the view that WRN might play an upstream role in response to DSBs at replication forks [38, 39]. WRN is required for activation of ATM as well as phosphorylation of downstream ATM substrates in cells with collapsed replication forks [39]. A recent finding by Ammazalorso et al., suggests that both ATM and ATR differentially regulate WRN to prevent accumulation of DSBs at stalled replication forks. WRN is directly phosphorylated by ATR and the suppression of ATR-mediated phosphorylation of WRN prevents proper accumulation of WRN in nuclear foci and co-localization with RPA, and causes breakage of stalled forks [40]. Contrary to this, inhibition of ATM kinase activity leads to retention of WRN in nuclear foci and impaired recruitment of RAD51 recombinase resulting in reduced viability after fork collapse [40].

WRN plays an important role in resolving Holiday junctions (HJs), which are formed as a recombination intermediate during HR. WRN could actively unwind HJ structures [41–43]. Using an isogenic WS cell line expressing a nuclear targeted bacterial HJ endonuclease, RusA, Rodriguez-Lopez et al. showed that HJ resolution by RusA restores DNA replication capacity in primary WS fibroblasts and enhances their proliferation. Also, RusA expression rescued hypersensitivity of WS fibroblasts cells to camptothecin, which induces formation of a DSB and fork collapse [44]. Thus, WRN is important *in vivo* in preventing accumulation of HJs. WRN also promotes the ATP-dependent translocation of HJs which are consistent with the model in which WRN prevents aberrant recombination events at sites of stalled replication forks by dissociating recombination intermediates [41, 44].

DSBs are also repaired by NHEJ pathway. Studies have shown extensive deletions at non homologous ends of the linear plasmids with incompatible ends when introduced into WS cells. Thus, it was predicted that WRN might suppress extensive nucleotide loss during NHEJ and prevent aberrant DNA repair potentially by stabilizing the broken DNA ends or by direct competition with other helicases or exonucleases [45]. The WRN protein interacts with the Ku 70/80 complex, and in turn the Ku complex stimulates the WRN exonuclease activity but does not affect the helicase activity [28, 29]. WRN also interacts with DNA-PK ζ in a Ku70/80 dependent manner. WRN protein is a target for DNA-PK phosphorylation *in vitro* and *in vivo*, which is important in regulating the different catalytic activities of WRN [46]. Thus, WRN, Ku70/80 and DNA-PK ζ form a trimeric complex in solution. Further, WRN displaces DNA-PK ζ from the DNA, raising the possibility of a direct involvement of WRN in DNA end processing [29]. Studies by Kusumoto et al., have shown that WRN physically and functionally interacts with the NHEJ factor XRCC4-DNA ligase IV complex (X4L4) which stimulates WRN exonuclease activity and not WRN helicase activity. Further, X4L4 is able to ligate a substrate processed by WRN exonuclease, suggesting the functional importance of this interaction [30]. These results suggest that WRN plays a role in regulating different stages of NHEJ pathway of DSB repair.

1.1.2 BLM in DSB repair—Cellular investigations of the cells with Bloom syndrome show an elevated level of several types of chromosomal aberrations, including breaks, quadriradials and translocations. The prominent feature of BS cells is highly elevated levels of the frequency of sister chromatid exchanges (SCEs) [47, 48]. BLM plays important roles at multiple steps of DSB repair, i.e., promotes strand resection step in complex with Exo1, disrupts Rad51 mediated nucleoprotein filament formation, and at later stages helps in dissolution of HJs by branch migration activity. Consistent with its roles in HR, BLM physically interacts with HR proteins Exo1, RAD51 and Rad51D, as well as with several other proteins involved in DNA repair such as Mus81, MLH1, MSH6, RPA and ATM [49–53].

BLM interacts with human exonuclease1 hExo1, and stimulates its nucleolytic activity which is involved in extensive resection of DSB ends at the initiation step of DSB repair by HR. Then the DNA ends resected by hExo1 and BLM are utilized by RAD51 to promote homologous recombination [54] RAD51 binds to the ssDNA resected ends of DSBs to form a nucleoprotein filament, followed by strand invasion leading to the formation of a displacement loop (D-loop). Studies have revealed two novel pro- and anti-recombination activities of the human BLM helicase at different stages [55]. In the early phase of HR, BLM disrupts the RAD51-ssDNA filament by dislodging human RAD51 protein from ssDNA in an ATPase dependent manner, thus preventing the formation of a D-loop [55]. Further, BLM may also act downstream of D-loop formation [56, 57]. HR can proceed downstream by several pathways. Two pathways, synthesis-dependent strand-annealing (SDSA) and double Holliday junction (DHJ) dissolution, result exclusively in the formation of non-crossover products. BLM has been implicated in affecting both of these processes [57, 58]. In one case, a D-loop may eventually be converted to a DHJ, and is then processed by dissolution of the HJ. However, SDSA requires the dissociation of a D-loop allowing complementary 3' ssDNA tails of the broken chromosome to anneal and be ligated following DNA repair gap filling. Bugreev et al. showed that BLM may promote SDSA by facilitating D-loop mediated DNA repair synthesis [55, 58].

Studies have also shown that BLM interacts physically and functionally with the type IA topoisomerase Topo III α , both localize to PML nuclear bodies and catalyzes a novel reaction in the resolution of recombination intermediates involving DHJ [59, 60]. This reaction gives rise exclusively to non-cross-over products which fits very well with the role of BLM as a suppressor of SCEs. The BLM-Topo III α pair is tightly associated with a third protein called BLAP75. Attenuation of BLAP75 levels by RNA interference destabilizes both BLM and Topo III α [61]. Biochemical analyses have revealed specific and direct interactions of BLAP75 with BLM and Topo III α and a strong enhancement of the BLM-Topo III α -mediated DHJ dissolution reaction by this novel protein [62, 63]. BLAP75 in conjunction with Topo III α greatly enhances the HJ unwinding activity of BLM. This functional interaction is highly specific, as the BLAP75-Topo III α pair has no effect on either WRN or *Escherichia coli* RecQ helicase activity, nor can *E. coli* Top3 substitute for Topo III α in the enhancement of the BLM helicase activity [64].

The evidence suggests that BLM also plays an important role in the repair of stalled or collapsed replication fork during the S-phase of the cell cycle. BS cells exhibit abnormal formation of replication intermediates formation, delayed Okazaki fragment maturation and a hypersensitivity to various inhibitors of replication [65, 66]. In response to hydroxyurea-induced replicative stress, BLM localizes to repair centers at collapsed replication forks which are dependent on ATM and ATR [67]. One potential role of BLM at the stalled replication fork is to promote the fork regression. As a result of HR-mediated restart/repair of a damaged replication fork, sister chromatids become covalently linked by HJs, which need to be resolved prior to mitosis. BLM is able to both bind and branch migrate synthetic

HJ [68]. It has been shown in *S. cerevisiae* that loss of Sgs1 results in the accumulation of HR-dependent replication intermediates that resembles HJs [69] suggesting that BLM might function in resolving HJs in a TopIII α and BLAP75 dependent manner [68, 70].

1.1.3 RECQL4 in DSB repair—The cytological investigations of the cells derived from RTS patients as well as the RECQL4 knockout mice show genomic instability and chromosomal abnormalities such as trisomy, aneuploidy and chromosomal rearrangements, suggesting a defect in the sister chromatid separations during recombination [71–75]. Similar kinds of chromosomal abnormalities have also been seen in *Drosophila* RECQL4 knockout cells [76]. These cellular features suggest a role for *RECQL4* gene in preventing tumorigenesis and maintaining genome integrity in humans.

Recent studies have suggested the active involvement of RECQL4 protein in the repair of DSBs. In *Drosophila*, RECQL4 knockout cells are hypersensitive to ionizing radiation [76]. Although primary fibroblasts from RTS patient show modest sensitivity towards ionizing radiations (IR), the patient derived RTS cells show deficiency in efficient repair of DSBs and accumulate higher levels of 53BP1 foci compared to normal fibroblasts [20]. Direct recruitment studies using laser confocal microscopy revealed that RECQL4 is efficiently recruited to the DSB sites and furthermore it showed that RECQL4 displayed distinct kinetics compared to WRN and BLM proteins [20]. After etoposide treatment, RECQL4 has also been shown to form complexes with Rad51 which is crucial for the repair of DSBs by the HR pathway [77]. In another study, in *Xenopus*, RECQL4 has been shown to be loaded onto the chromatin adjacent to Ku heterodimer binding sites in response to DSBs suggesting its possible involvement in the NHEJ pathway [78]. This emerging evidence suggests the possible involvement of RECQL4 in both the HR and NHEJ pathways of DSB repair. However, the detailed biochemical mechanism of RECQL4's involvement in DSB repair is still not known. A possibility is that similar to WRN and BLM helicases, RECQL4 could function in resolving aberrant DNA structures formed during DNA replication and recombination repair processes and facilitate the loading of other repair factors at the site of the DSB.

1.1.4 RECQL1 in DSB repair—Studies have shown that the RECQL1 depleted human cells are sensitive to IR or camptothecin and the cells show a high level of spontaneous γ -H2AX foci and elevated SCE, indicating an accumulation of DSBs. This is also corroborated by the fact that RECQL1 physically interacts with RAD51 suggesting its prominent roles in the HR pathway [79]. Consistent with the role of RECQL1 in HR, RECQL1 also possess ATP-dependent HJ branch migration activity [80]. Biochemically, RECQL1 promotes both the three-stranded and four-stranded branch migration [81]. A specific feature of RECQL1-catalyzed branch migration is a strong preference towards the 3'→5' polarity in both the three and four-stranded reactions, which distinguishes RECQL1 from other known branch migration proteins such as BLM helicase and RAD54 which show no significant preference in branch migration directionality. This unique 3'→5' branch migration activity allows RECQL1 to specifically disrupt recombination intermediates (D-loops) formed by invasion of tailed DNA with the 5'-protruding ends. These D-loops, in contrast to the D-loops formed by invasion of tailed DNA, with the 3'-protruding ends cannot be readily extended by DNA polymerase and therefore may represent illegitimate recombination intermediates during DSB repair. Thus, RECQL1 branch migration activity may prevent accumulation of these unconventional and potentially toxic intermediates *in vivo*.

Another possible function of RECQL1 could be in a HR dependent restart of collapsed replication forks. DSBs with 5'-protruding ends can be generated when incoming replication forks encounter nicks on the DNA template strand or they may also be produced by

endonucleolytic cleavage of stalled replication forks. In both scenarios, invasion of non-processed 5'-tailed DNA into homologous duplex DNA may result in a failed replication restart. In such cases, RECQL1 may specifically dissociate non-productive 5'-invaded loops, allowing processing of 5'-tailed DNA ends into 3'-tailed ends by exonucleases followed by their invasion into homologous dsDNA by RAD51.

1.1.5 RECQL5 in DSB repair—Recent studies in mice have shown that deletion of RECQL5 results in increased susceptibility to cancer. RECQL5 deleted cells exhibit elevated frequencies of spontaneous DSBs and are prone to the accumulation of gross chromosomal rearrangements in response to replication stress [82]. Biochemically, human RECQL5 physically interacts with RAD51, and catalyzes the disruption of RAD51 mediated presynaptic filament formation [83]. These results suggest an anti-recombinogenic property of RECQL5 and the involvement of RECQL5 in minimizing chromosomal rearrangements and tumorigenesis by suppressing the accumulation of DSBs and attenuating HR [82]. This is similar to the BLM function in suppressing HR by inhibiting the Rad51 presynthetic filament (discussed above). However, BLM in addition to providing a presynaptic disruptive function also acts to resolve a late HR intermediate in favor of gene conversions [55]. Importantly, both mechanisms are required for tumor suppression. RECQL5 also interacts functionally with the MRN complex and RECQL5 specifically inhibits the 3'→5' exonuclease activity of Mre11. Moreover, the MRN complex is required for the recruitment of RECQL5 to sites of DNA damage to regulate DNA repair [21].

2.1 Telomere: Structure and Maintenance

Telomere maintenance is another very important aspect for the preservation of genome stability. The RecQ helicases play very significant roles in replication, recombination and repair at the telomere.

The “end replication problem”, caused by the unidirectional nature of DNA polymerases, restricts the enzymes involved in DNA replication process from continuing the DNA synthesis to the ends of the chromosome [84–86]. Eukaryotic cells evolved a unique solution to the end replication problem by creating a special structure known as the telomere [87]. Telomeres are situated at the ends of each eukaryotic linear chromosomes and prevent chromosome termini from being recognized as broken DNA ends (*i.e.*, DSBs). In mammals, telomeres are composed of double-stranded tandem repeat sequences, followed by a single-stranded short 3'-overhang and telomere-associated proteins. Telomeres normally exist in a loop structure which is packaged together into either three-stranded DNA displacement loops, (D-loops) or telomere-loops (T-loops). [88]. Disruption of the D-loop and subsequent exposure of the 3'-overhang represent an uncapped state of telomeres. 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 [89–91]. Telomere attrition is frequently associated with aging [92] and premature aging syndromes [93]. Several factors, including telomerase, the shelterin complex, and telomere structures are critical for telomere maintenance.

Telomerase is a crucial component of telomeres that maintains the telomere length. It contains two core components, telomerase reverse transcriptase (TERT in mammals or Est2 in *S. cerevisiae*) and telomerase RNA. Telomerase is recruited to the 3' telomeric overhang after DNA replication where it extends the telomeric repeat using its integral telomerase RNA as a template. Although telomerase activity is essential in preventing replication-dependent telomere loss in highly proliferative and cancer cells, most human somatic cells possess low or undetectable telomerase activity. This results in replication-associated

telomere shortening and progressive restriction of the replicative potential of cells grown in culture [94].

The telomere nucleoprotein complex, the shelterin complex, includes telomere-specific binding proteins and their associated proteins [95]. In mammals, this complex includes TRF1 and TRF2, proteins that bind to the dsDNA telomeric region, and a protein that binds to the ssDNA telomeric overhang, POT1, as well as their associated proteins TIN2, TPP1. The telomere protein complex controls telomere length *in cis* by modulating the action of telomerase at the ends of individual telomeres. Telomeres that are severely or completely stripped of the protective telomere protein complex evoke a DNA damage response [96–98]. Specifically, uncapped telomeres also become the substrates for HR or NHEJ repair, thus leading to inappropriate and deleterious chromosome end fusion events.

2.2 RecQ helicases in telomere maintenance

RecQ helicases, especially WRN, are known to play significant roles in proper maintenance of telomeres. A strong argument for the function of WRN in telomere processing is that the WRN and TERT deficient mice, during late generations, show clinical features resembling human WRN patients or premature aging [99]. Mice deficient in WRN alone have no phenotype. *In vivo* gene specific repair studies have shown that the extent and rate of telomeric repair is lower in WS patients [100]. This notion is further supported by accelerated telomere loss displayed in WS cells [101]. Another well studied RecQ helicase, BLM, was detected at the telomeres of ALT cells by a mass spectrometry study and has been implicated in telomere maintenance by several research groups [93, 102–104]. Recently, we have found a novel role of RECQL4 in telomere metabolism (Avik Ghosh et al., submitted for publication). Additionally, RECQL5 knockout mice show no increased susceptibility to cancer till late in life, indicating a possible role of RECQL5 in telomere maintenance. Here, we will discuss the roles for WRN and other RecQ helicases in some important processes at telomeres. Roles of RecQ helicases in telomere replication and repair are shown in Fig. 3.

2.2.1 Telomeric DNA Replication—RecQ helicases have important functions in resolving potential impediments in telomeric DNA replication that can stall or block the replication forks (see Fig. 3). Early evidence of the involvement of WRN in replication comes from the fact that WS cells are very sensitive to agents that cause replication fork block and show an extended S-phase [105]. WRN was found in telomeres of human cells and this association increases after replication stress indicating a role for WRN in telomeric DNA replication. However, experiments showed that WRN was associated with only 5% of telomeric foci in S-phase fibroblasts suggesting that it might not participate in the general telomeric replication process [93]. It is more likely that WRN is recruited to replicating telomeres in response to replication stress. Supporting this hypothesis, WRN suppresses telomeric instability caused by chromium (VI) induced DNA replication stress [106]. The CO-FISH studies by Crabbe et al. also suggest that the requirement of WRN as a part of an alternative mechanism to resolve relatively rare, but lethal, events during telomere replication [107]. Recent studies from the De Lange group suggest that the recruitment of BLM by TRF1 is an important step in telomere replication and is required to repress the fragile-telomere phenotype [104]. We have recently found that RECQL4 also associates with telomeres during S phase and depletion of this protein results in telomere replication defects including fragile telomeres and telomere sister chromatid exchanges (Avik Ghosh et al., submitted for publication).

Replication fork terminations within telomeres are particularly damaging due to the absence of any replication origins resulting in vast stretches of unreplicated telomeric DNA.

Replication of telomeric DNA requires the dissociation of D-loop/T-loop structures. WRN, BLM and RECQL4 unwind the D-loop structures to release the invading strand *in vitro* [103, 108, 109]. TRF2, in particular, interacts physically and enhances the helicase activity of WRN, BLM and RECQL4 at telomeric D-loop structures [110]. The telomeric single strand binding protein POT1 also improves the D-loop unwinding ability of these three helicases *in vitro* [111]. DNA-PKcs, a telomere associated protein, stimulates WRN helicase activity on telomeric D-loop substrates. In addition, the length of telomeric G-tails decreases in DNA-PKcs knockdown cells, and this phenotype is reversed by overexpression of WRN helicase [112].

Another potential block to the replication of the invading telomeric DNA strand could be the formation of G-quadruplex (G4) structures. *In vitro* studies confirmed that the formation of these structures in telomeric (TTAGGG)_n strands and bimolecular G4 structures are favored substrates for WRN and BLM [113]. Furthermore, Kamath-Loeb et al reported that WRN can prevent replication stalling at G4 DNA [114]. POT1 is also known to resolve the G4 structures and it interacts with WRN and BLM. Thus, these three proteins could work together to dissociate G-quadruplex structures in telomeres (Fig. 3).

2.2.2 Alternative Lengthening of Telomeres—Rare cells emerge from the crisis of telomere shortening, and these cells employ an alternative telomere maintenance strategy, the alternative lengthening of telomere (ALT) pathways. This pathway involves multiple telomere binding proteins and recombination. In budding yeast, RecQ helicase Sgs1 functions in a recombination dependent ALT pathway [115]. When critically short, telomeres undergo recombination to try to restore their telomeric length, Sgs1 helps resolve these recombination intermediates. In humans it was shown that WRN and BLM can partially substitute the function of yeast Sgs1 in type II ALT [115, 116].

In human cell lines, a fraction of telomeric DNA is maintained by the ALT mechanism and these regions co-localize with WRN [93]. *In vitro* studies indicate that recombination intermediates, such as a four-way Holliday junction (HJ) and D-loops, are excellent substrates for WRN and BLM. As mentioned before, these two RecQ helicases also function in resolving recombination intermediates that arise during DNA repair. WRN also has branch migration activity on telomeric strands and this function is stimulated by RPA [109]. Additionally, WRN interacts physically with the Ku 70/80 heterodimer, which suppresses recombination at telomeres [117]. Thus, WRN can function with Ku in suppressing the telomeric recombination intermediates.

2.3 Telomere damage and environmental factors

Ultraviolet (UV) light, a known carcinogen, is a major source of exogenous DNA damage. The most pronounced DNA damage caused by UV-B light are cyclobutane pyrimidine dimers (CPD) [118]. In a recent study, the Brash group used an “immunoprecipitation of DNA damage” (IPoD) technique to measure the UVC induced DNA damage at different regions of genomic DNA. UVC minimizes the introduction of photosensitized oxidative DNA damage that accompanies UVB. Their results suggest that the repeated TTAGGG sequences in the telomeres render this region seven times more sensitive to UVC than two other tested regions of the genome. As described in the next section, this region is also more sensitive to oxidative damage. Moreover, 48 hours after the UVC treatment less than 10% of the damage was repaired in the telomere region while approximately 70% and 40% CPD lesions were removed from *p53* and 28S regions, respectively [119].

Hexavalent chromium (Cr(VI)) is an important source of DNA replication stress and many respiratory problems including lung cancer are strongly linked to the inhalation of Cr(VI) particles in the occupational setting [106]. Although, short telomeres are associated with

increased risk for lung cancer, Cr(VI) exposure does not significantly alter mean telomere lengths [120–122]. However, in cells Cr (IV) is reduced to Cr (III) which reacts with DNA and produces a wide array of lesions. Oxygen radical mediated oxidative DNA damage could be caused by Cr(III)-complexes targeting the guanine (G) tracts in telomeres as hot-spot for oxidative DNA damage. Moreover, *in vitro* DNA polymerase arrest induced by Cr (VI) treatment is most potent at templates with G runs [123, 124]. Recent studies have shown that telomeric abnormalities such as sister telomere loss and sister telomere fusion is elevated in Cr (VI) treated lung cancer cells and human skin fibroblasts. Interestingly, expression of telomerase was able to protect the human skin fibroblasts from these abnormalities.

2.3.1 Repair of oxidative damage—Telomeric DNA contains repetitive guanine triplets and thus is hypersensitive to DNA damage induced by oxidative stress [125, 126]. The telomeric region (TTAGGG) of genomic DNA isolated from mouse kidney contains more Fpg-sensitive oxidatively damaged sites than in a minisatellite (TG) region [127]. *In vitro* analysis has shown that telomeric DNA is prone to oxidative damage because of the triple guanines (G). Guanine has the lowest oxidation potential among the nucleobases and the GGG sequence has 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 the association between oxidative damage and telomere shortening [128, 129]. Oxidative damage is repaired by the base excision repair (BER) process. WRN is believed to take part in BER and physically interacts with several proteins involved in BER [5]. Oxidative damage can result in base lesions and DSBs in telomeres and WRN is also implicated in the DSB repair process. Recently, we have observed that WRN and BLM interact with the *in vitro* D-loop structures containing 8-oxodG lesion and unwind these substrates more efficiently than the undamaged D-loops [108]. However, still a great deal of work needs to be done to assess the exact role of WRN and other RecQ helicases in the repair of oxidative damage in telomeres.

Conclusions and future perspectives

The RecQ helicases are important for the preservation of genome stability. The presence of multiple forms of RecQ helicases in higher eukaryotes could be an adaptive feature to ensure proper surveillance of the genome against the harmful effects of various environmental insults. The RecQ helicases have redundant and/or non-redundant functions depending on type of DNA damage and DNA repair pathways. Therefore, in the future it would be very interesting to understand how these RecQ helicases cooperate among themselves and with other DNA repair proteins.

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Fig. 1. Involvement of RecQ helicases in maintenance of genomic integrity. Schematic diagram summarizing the different kinds of DNA lesions caused by various environmental factors. The association of different RecQ helicases (RecQ1, WRN, BLM, RecQL4, and RecQL5) with replication, base excision repair (BER), double strand break repair (DSBR), transcription, and telomere maintenance are shown by arrows. Abbreviations: G^{ox}, 8-oxoG; AP-apurinic or apyrimidinic; CPD-cyclobutane pyrimidine dimer.

Fig. 2.

RecQ helicases are involved in multiple steps of the DNA double strand break repair pathways. The members of the RecQ helicases interact with various key proteins involved in different steps of both the homologous recombination (HR) pathway and the non-homologous end-joining (NHEJ) pathway of DSB repair (see text for details).

Fig. 3.
Role of different RecQ helicases in replication, recombination, and repair processes at telomeres (see text for details)