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The role of RecQ helicases in non-homologous end-joining

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Abstract

DNA double-strand breaks are highly toxic DNA lesions that cause genomic instability, if not efficiently repaired. RecQ helicases are a family of highly conserved proteins that maintain genomic stability through their important roles in several DNA repair pathways, including DNA double-strand break repair. Double-strand breaks can be repaired by homologous recombination (HR) using sister chromatids as templates to facilitate precise DNA repair, or by an HR-independent mechanism known as non-homologous end-joining (NHEJ) (error-prone). NHEJ is a non-templated DNA repair process, in which DNA termini are directly ligated. Canonical NHEJ requires DNA-PKcs and Ku70/80, while alternative NHEJ pathways are DNA-PKcs and Ku70/80 independent. This review discusses the role of RecQ helicases in NHEJ, alternative (or back-up) NHEJ (B-NHEJ) and microhomology-mediated end-joining (MMEJ) in V(D)J recombination, class switch recombination and telomere maintenance.

Keywords

Alternative end-joining; Ku70/80; microhomology-mediated end-joining; non-homologous end-joining; RecQ helicases; telomere

Introduction

The stability of mitochondrial and genomic DNA is critical for viability of eukaryotic cells, and survival of organisms and species. DNA repair pathways exist to counterbalance the adverse impact of nuclear and mitochondrial DNA damage, generated by exogenous and endogenous DNA damaging agents. Such damage results from exposure to genotoxic chemicals, ultraviolet light (UV), ionizing radiation (IR) and reactive oxygen species (ROS). ROS are a considerable source of endogenous DNA damage, such as DNA strand breaks and oxidized DNA bases. ROS are continuously produced in the cell, particularly because they are a byproduct of mitochondrial oxidative phosphorylation. Defects in DNA repair can

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lead to an increased load of persistent DNA lesions. Dysfunctional DNA repair is strongly associated with aging and increased risk of age-related disease. Unrepaired DNA damage is thought to contribute to the occurrence of cancer and neurodegenerative disease, including Alzheimer's disease and Huntington's disease (Jeppesen *et al.*, 2011).

Eukaryotic organisms possess a sophisticated network of DNA repair pathways, some of which provide redundant or overlapping DNA repair functions. Many DNA repair enzymes are multifunctional, playing roles in DNA repair and DNA replication or recombination, as well as roles in development and gene expression. The main DNA excision repair pathways are base excision repair (BER), mismatch repair (MMR) and nucleotide excision repair (NER). The main DNA double strand-break repair pathways are non-homologous end-joining (NHEJ) and homologous recombination (HR).

The RecQ protein family is a highly conserved group of DNA helicases that play essential roles in transcription, DNA replication, DNA recombination and DNA repair. *Escherichia coli* RecQ is the only RecQ helicase expressed in the bacterial cell (Bernstein & Keck, 2003). However, five RecQ helicases are expressed in human cells: RECQL1, BLM, WRN, RECQL4 and RECQL5 (Singh *et al.*, 2009). Mutations in *BLM*, *WRN* and *RECQL4* cause the inherited disorders, such as Bloom syndrome (BS) (Online Mendelian Inheritance in Man (OMIM) #210900), Werner syndrome (WS) (OMIM #277700) and Rothmund–Thomson syndrome (RTS) (OMIM #268400), respectively. Although these diseases are clinically distinct, they share some features, including cancer susceptibility and genomic instability. WS and RTS are segmental progerias, in that affected patients manifest many, but not all, of the typical features of aging. Defects in *RECQL1* and *RECQL5*, which code for the other two main RecQ helicases, have not yet been linked to human disease (Singh *et al.*, 2009). This review focuses on the role of RecQ helicases in NHEJ, including canonical DNA-PK-mediated NHEJ (C-NHEJ), alternative (or back-up) NHEJ (B-NHEJ) and microhomology-mediated end-joining (MMEJ) in V(D)J recombination, class switch recombination and telomere maintenance.

DNA repair, aging and cancer

Accumulation of DNA damage and defects in DNA repair are linked to disease, poor health, premature aging and cancer susceptibility (Bohr, 2008; Brosh, 2013; Maynard *et al.*, 2009, 2014; Wilson *et al.*, 2008). For example, rare inherited progeroid syndromes, that mimic normal physiological aging at an early stage in the life of the patient, are caused by mutations in genes encoding proteins that have roles in DNA repair. Defects in DNA repair cause BS, WS, RTS, Cockayne syndrome (CS) (OMIM #133540) and Trichothiodystrophy (TTD) (OMIM #601675) (Croteau *et al.*, 2014; Kitao *et al.*, 1999; Siitonen *et al.*, 2009; Weeda *et al.*, 1997; Yu *et al.*, 1996). Mutations in the lamin A/C gene (*LMNA*) cause Hutchinson–Gilford progeria (HGPS OMIM #176670), autosomal dominant Emery–Dreifuss muscular dystrophy (OMIM #7894480), restrictive dermopathy (OMIM #275210) and atypical WS (Bione *et al.*, 1994; Chen *et al.*, 2003b; De Sandre-Giovannoli *et al.*, 2003; Navarro *et al.*, 2004). Mutations in *CSA* and *CSB* genes, the products of which promote NER, cause CS, which is characterized by premature aging. Mice depleted in either *Ku70* or *Ku80* genes, that are both essential in the NHEJ pathway, develop aging-like pathology

prematurely (Gu *et al.*, 1997; Nussenzweig *et al.*, 1996). Evidence that HR plays a major role in aging is limited; however, defects in BLM, a RecQ helicase with roles in DSB repair and DNA replication, have been found to be associated with increased cancer risk (Pagon *et al.*, 1993). The molecular and cellular phenotypes in patients with premature aging syndromes have provided considerable insight into mechanisms involved in normal aging.

Deficiencies in BER, MMR, NER and NHEJ are thought to contribute to neurological dysfunction associated with the neurodegenerative diseases, such as Alzheimer's disease and Huntington's disease (Jeppesen *et al.*, 2011). Weissman and colleagues (2007) showed that BER capacity is lower in postmortem brain tissue from patients with sporadic Alzheimer's disease, relative to unaffected controls. In addition, the efficiency of BER appeared lower in differentiated neurons (postmitotic cells), relative to undifferentiated neurons (Sykora *et al.*, 2013). Moreover, some recent evidence suggests that defective BER or MMR may contribute to the pathology of Huntington's disease (Møllersen *et al.*, 2010; Owen *et al.*, 2005; Wheeler *et al.*, 2003).

Characteristics of human RecQ helicases

Bloom syndrome is caused by mutations in the *BLM* gene. Patients with BS display short stature, immunodeficiency and predisposition to cancer. BLM is a DNA-stimulated ATPase and ATP-dependent 3′ to 5′ DNA helicase and contains strand-annealing activity. Some mutations linked to BS map to helicase motifs and thus can disable BLM helicase activity (Guo *et al.*, 2007; Rong *et al.*, 2000). BLM interacts with several HR proteins including RAD50, MRE11, RAD51 and BRCA1 (Croteau *et al.*, 2014; Davalos & Campisi, 2003; Ding *et al.*, 2009; Franchitto & Pichierri, 2002; Wang *et al.*, 2000). The single-stranded DNA binding protein, replication protein A (RPA), interacts directly with BLM and stimulates its helicase activity (Brosh *et al.*, 2000). BLM also interacts with WRN, FEN1, EXO1, phosphorylated histone H2AX (γ-H2AX), Fanconi anemia group D2 (FANCD2), Ataxia telangiectasia mutated (ATM), telomeric repeat-binding factor 2 (TRF2), Casp3 and p53 (Freire *et al.*, 2001; Nimonkar *et al.*, 2008; Opresko *et al.*, 2002; Sengupta *et al.*, 2003; von Kobbe *et al.*, 2002; Wang & Bambara, 2005; Wang *et al.*, 2001). BLM binds preferentially to single-stranded DNA, Y-shaped DNA and double Holliday Junctions (dHJ) (Mohaghegh *et al.*, 2001). The yeast BLM ortholog Sgs1 stimulates helicase/exonuclease DNA2 during single-stranded DNA annealing (Zhu *et al.*, 2008). Human BLM does not play a major role in the canonical (C-)NHEJ or V(D)J recombination (Chen *et al.*, 2003a; So *et al.*, 2004).

Werner syndrome is caused by mutations in the *WRN* gene. Patients with WS display cancer susceptibility, short stature, alopecia, atrophic skin, thin gray hair, type II diabetes, osteoporosis, cataracts, arteriosclerosis and atherosclerosis, indicating that WS is a segmental progeria (Yu *et al.*, 1994). WRN possesses intrinsic 3′ to 5′ DNA helicase activity, DNA single-strand DNA annealing activity and 3′ to 5′ exonuclease activity. WRN binds preferentially to single-stranded DNA, Y-shaped DNA, G-quadruplex and dHJ (Brosh *et al.*, 2001; Croteau *et al.*, 2014). In the presence of single-stranded DNA binding protein (SSB) of *E. coli*, WRN helicase can unwind short DNA duplexes (<53 base pairs) (Shen *et al.*, 1998), whereas, human SSB protein, RPA, stimulates WRN to catalyze unwinding of

long duplex DNA substrates up to 849 base pairs (Brosh *et al.*, 1999; Shen *et al.*, 1998). The 3′ to 5′ exonuclease activity of WRN is stimulated by Ku70/80 (Cooper *et al.*, 2000). WRN contains a nuclear localization signal in its C-terminal region as well as a nucleolar localization signal (Marciniak *et al.*, 1998). In response to DNA damage, WRN translocates out of the nucleolus to perform its enzymatic function (Indig *et al.*, 2012). WRN interacts with RAD52, NBS1, Ku70/80, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Ligase IV/XRCC4 (Baynton *et al.*, 2003; Cheng *et al.*, 2004; Karmakar *et al.*, 2002a, b; Kusumoto *et al.*, 2008), FEN1, PARP1, BLM, TRF1, TRF2 and p53 (Blander *et al.*, 1999; Opresko *et al.*, 2002, 2004; von Kobbe *et al.*, 2002, 2003, 2004a). In cells lacking WRN, the frequency of chromosomal translocations increases (Chen *et al.*, 2003a).

RECQL4 has been less extensively studied than BLM and WRN. Defects in *RECQL4* are associated with three distinct clinical diseases, Rothmund–Thomson Syndrome (RTS), Rapadilino (RAPA) (OMIM #266280) and Baller–Gerold syndrome (BGS) (OMIM #218600). Of those patients with RTS, two-thirds have mutations in the *RECQL4* gene while the remainder have mutations in gene(s) yet to be identified (Wang *et al.*, 2003b). Patient with either RTS or RAPA are at increased risk for osteosarcomas and lymphomas (Larizza *et al.*, 2010; Siitonen *et al.*, 2009; Wang *et al.*, 2003b).

RECQL4 is a 3′ to 5′ helicase and stimulates DNA strand displacement in the presence of ATP (Macris *et al.*, 2006). RECQL4 function is regulated in part by interaction with RPA and BLM. RPA stimulates the RECQL4 helicase activity by approximately 2-fold on forked substrates (Rossi *et al.*, 2010). RECQL4 and BLM function cooperatively at forked substrates *in vitro*, and interact physically during S-phase (Singh *et al*., 2012). WRN, RECQL4 and RECQL5 promote repair of ssDNA breaks and oxidatively damaged DNA bases (Szekely *et al.*, 2005; Tadokoro *et al.*, 2012; von Kobbe *et al.*, 2004b; Werner *et al.*, 2006; Woo *et al.*, 2006). RTS cells show an accumulation of single stranded DNA breaks after peroxide treatment. RECQL4 interacts physically with several BER proteins, stimulates APE1 and FEN1 activities and modulates POLβ ligase activity (Schurman *et al.*, 2009). RECQL4 functions in telomeric maintenance and interacts with the shelterin proteins TRF1 and TRF2. RECQL4 also interacts physically with PARP1, XPA and WRN (Croteau *et al.*, 2012; Fan & Luo, 2008; Ghosh *et al.*, 2012; Petkovic *et al.*, 2005). While RECLQ4 is known to interact with and modulate the activity of repair proteins, it also plays a specialized role in DNA replication initiation (Capp *et al.*, 2009; Collart *et al.*, 2013; Thangavel *et al*., 2010; Wu *et al.*, 2008a; Xu *et al.*, 2009a,b).

RECQL1 is the most abundant RecQ helicase (Kitao *et al.*, 1998). The RECQL1 and WRN 3′ to 5′ DNA helicase activities unwind the leading strand of forked DNA substrates, while RECQL4 and RECQL5β only unwind the lagging strand (Popuri *et al.*, 2012a). RECQL1 alone is able to unwind short DNA duplexes (<110 base pairs), whereas it requires the presence of RPA to unwind longer substrates (501 base pairs) (Cui *et al.*, 2003, 2004). RECQL1 is thought to stimulate PARP1-mediated DNA replication restart (Berti *et al.*, 2013). Defects in RECQL1 lead to hyper-phosphorylation of RPA32 and activation of CHK1 (Popuri *et al.*, 2012a). DSBs and sister chromatid exchange (SCE) are more pronounced in RECQL1-deficient mouse embryo fibroblasts (Sharma & Brosh, 2007). RECQL1 also appears to be an important component in the cellular response to oxidative

damage, as evidenced by the observation that it is rapidly recruited to oxidative DNA lesions and that RECQL1-deficient cells are sensitive to oxidative damage (Sharma *et al.*, 2012).

RECQL5 encodes three RecQ isoforms, RECQL5α, RECQL5β and RECQL5γ. RECQL5α has strand annealing activity (Ren *et al.*, 2008), RECQL5β has 3′ to 5′ DNA helicase activity and ATPase activity, and no biochemical functions have been attributed to RECQL5γ. RPA stimulates RECQL5β helicase activity but inhibits RECQL5β ssDNA-annealing activity (Garcia *et al.*, 2004). RECQL5β is thought to be active during G0 and G1, and may play significant roles in DNA replication, DNA recombination and RNA transcription (Aygün *et al.*, 2008; Kawabe *et al.*, 2000; Kitao *et al.*, 1998; Zheng *et al.*, 2009). RECQL5β may play significant roles in HR, BER and interstrand cross-link repair (Ramamoorthy *et al.*, 2013; Tadokoro *et al.*, 2012; Zheng *et al.*, 2009). RECQL5β directly interacts with RAD51, thereby inhibiting RAD51-mediated D-loop formation (Schwendener *et al.*, 2010). RECQL5β and RECQL1 helicases lack the winged-helix motif, which plays an essential role in DNA unwinding. In addition, the RECQL5β helicase inefficiently resolves dHJ (Croteau *et al.*, 2014; Garcia *et al.*, 2004).

RECQL5β is constitutively expressed in all cell types throughout the cell cycle, while expression of WRN, BLM and RECQL4 is cell cycle-dependent and tissue-specific (Kawabe *et al.*, 2000; Sanz *et al.*, 2000). The cell cycle-independent expression of RECQL5β likely reflects the important role that RECQL5β plays in transcription in resting cells, via its interaction with the C-terminal domain of RNA polymerase II (RNA pol II) (Aygün *et al.*, 2008, 2009; Izumikawa *et al.*, 2008). Interestingly, RECQL5β has two RNA polymerase II interaction motifs, the KIX and SRI domains, which both mediate binding to RNA pol II. A recent crystal structure analysis suggests that the KIX domain on RECQL5 and the transcription elongation factor, TFIIS, share overlapping binding sites on RNA pol II, such that when RECQL5β occupies the site, TFIIS cannot stimulate transcription elongation (Kassube *et al.*, 2013). Thus, the absence of RECQL5 leads to transcriptional stress across the genome, underscoring RECQL5β's importance during transcription (Saponaro *et al.*, 2014).

Canonical NHEJ

Canonical NHEJ (C-NHEJ) is one of the error-prone DSB repair mechanisms and it is constitutively active throughout the cell cycle (Helleday *et al.*, 2007; Rothkamm & Löbrich, 2003). Core C-NHEJ factors are Ku70/80, DNA-PKcs, XLF and Ligase IV/XRCC4 (Figure 1) (Weterings & Chen, 2007). Ku70/80 has a high affinity for DNA DSBs (Walker *et al.*, 2001) and forms a stable complex with DNA-PKcs that protects DSBs from degradation (Weterings *et al.*, 2003). DNA-PKcs, a PI3 kinase, binds the Ku70/80-DSB complex, which triggers DNA-PKcs autophosphorylation and phosphorylation of additional DSB repair factors (Ding *et al.*, 2003; Reddy *et al.*, 2004). DNA termini are resected and/or extended by DNA polymerases Pol μ and Pol λ (Nick McElhinny *et al.*, 2005), and XLF and the Ligase IV/XRCC4 complex are recruited to the DNA lesion, where XLF interacts with XRCC4 and stimulates re-adenylation of Ligase IV (Ahnesorg *et al.*, 2006; Riballo *et al.*, 2009; Yano *et al.*, 2008). Ligase IV/XRCC4 then ligates the remaining nick. DSBs that require no resection (ligatable DNA ends) are repaired by Ku70/80 and XRCC4/Ligase IV/XLF, independent of

DNA-PKcs (Mari *et al.*, 2006; Reynolds *et al.*, 2012). Extensive work on WRN in C-NHEJ, demonstrates that WRN physically and functionally interacts with several C-NHEJ repair proteins (Karmakar *et al.*, 2002b; Kusumoto-Matsuo *et al.*, 2010, 2014). Ku70/80 and Ligase IV/XRCC4 stimulate WRN exonuclease but not WRN helicase activity (Cooper *et al.*, 2000; Kusumoto *et al.*, 2008; Li & Comai, 2001), clear demonstrations of how protein interactions dictate specificity of enzyme activities. Besides WRN, RECQL1 plays a major role in the initial steps of C-NHEJ. This is evidenced by data showing that it physically and functionally interacts with the heterodimer Ku70/80. Moreover, depletion of RECQL1 resulted in reduced end joining in cell-free extracts (Parvathaneni *et al.*, 2013).

V(D)J recombination

C-NHEJ contributes to V(D)J recombination, the process by which genes encoding immunoglobulin and T-cell receptors are diversified in B and T cells in the bone marrow (Gellert, 2002). The recombination-activating genes 1 and 2 (RAG1 and RAG2) recognize and cleave a signal sequence in these genes, after which the cleaved DNA rearranges into a DNA hairpin that bridges the newly-formed DNA termini. This structure is resolved in a manner that generates diversity at the cleavage site by a process that requires enzymes that catalyze C-NHEJ, namely Artemis and CtIP (Bothmer *et al.*, 2013; Ma *et al.*, 2002; Malu *et al.*, 2012; Weterings *et al.*, 2009). WRN, BLM, RECQL4 and RECQL1 are differentially up-regulated to guarantee genomic stability in proliferating B cells (Kawabe *et al.*, 2000). The different expression levels of the various helicases often reflect ongoing cell cycle-dependent cellular processes. For example, BLM is upregulated during replication to suppress sister chromatid exchange. RECQL1 expression is reduced during G0 phase, due to distinctive roles from BLM and RECQL4 during cellular proliferation and maintenance of chromosomal stability (Kawabe *et al.*, 2000; Popuri *et al.*, 2008; Sharma & Brosh, 2007; Thangavel *et al.*, 2010). In resting B cells, RECQL4 and WRN have a low level of expression, in contrast to their expression levels in proliferating B cells (S-phase). Increased levels of RECQL4 and WRN during S-phase may reflect their important roles in replication and could promote genomic stability by way of their roles in promoting DSB repair (Kawabe *et al.*, 2000; Singh *et al.*, 2010).

Alternative NHEJ

The alternative (i.e. back-up) NHEJ (B-NHEJ), substitutes for C-NHEJ when C-NHEJ is defective (Fattah *et al.*, 2010; Ma *et al.*, 2003; Wang *et al.*, 2003a). WRN is the only RecQ helicase thought to contribute to B-NHEJ (Sallmyr *et al.*, 2008). B-NHEJ is error-prone and independent of DNA homology for templating DSB repair (Mansour *et al.*, 2010). It has been suggested that B-NHEJ is more error-prone than C-NHEJ because it is slower in the repair of DNA (Durante *et al.*, 2013). Consistent with this idea, cells from mice deleted in the genes *Ku70*, *Ku80* or *Xrcc4* have higher rates of chromosomal translocations than wild-type control (Difilippantonio *et al.*, 2000; Ferguson *et al.*, 2000; Simsek & Jasin, 2010). B-NHEJ is active throughout the cell cycle, although it appears to be higher during G2 in cells that lack C-NHEJ (Wu *et al.*, 2008b). C-NHEJ and B-NHEJ may be simultaneous/parallel pathways in normal cells; it is not clear whether the pathways work in tandem, or whether B-NHEJ primarily acts as a backup system when C-NHEJ is compromised. B-NHEJ

enzymes include MRE11 of the MRE11-RAD50-NBS1 (MRN) complex, PARP1, WRN, Ligase I and DNA polymerase θ (Pol θ), a polymerase of the Y family (Figure 1) (Parsons *et al.*, 2005; Paull & Gellert, 1998; Sallmyr *et al.*, 2008; Simsek *et al.*, 2011). WRN helicase, exonuclease and single-stranded DNA annealing activities may contribute to B-NHEJ. Pol θ extends mismatched primer termini and can bypass abasic sites (Chan *et al.*, 2010; Hogg *et al.*, 2012). The contribution of the enzyme CtBP-interacting protein (CtIP) to B-NHEJ is still not understood, while its role in microhomology-mediated end-joining (MMEJ) is more clear (Durante *et al.*, 2013). A number of studies have used expression of oncogenic BCR-ABL kinase, a fusion protein linked to chronic myeloid leukemia (CML), as a tool to inhibit C-NHEJ and study B-NHEJ (Sallmyr *et al.*, 2008; Sattler *et al.*, 2000). For example, CML BCR-ABL cells show a higher expression levels of WRN and ligase III, as well as residual DNA breaks and large deletions, which indicates active roles for these enzymes in B-NHEJ (Sallmyr *et al.*, 2008; Slupianek *et al.*, 2011). Moreover, downregulation of WRN and Ligase III in CML cell lines resulted in accumulation of unrepaired DNA.

Microhomology-mediated end-joining

Microhomology-mediated end-joining (MMEJ) is an error-prone DNA repair mechanism targeted to long stretches of ssDNA, and thought to be more mutagenic than C-NHEJ and B-NHEJ (Ma *et al.*, 2003). MMEJ does not require Ku70/80, DNA-PKcs or Ligase IV/XRCC4, but does require BLM (Nussenzweig & Nussenzweig, 2007; Yu & Gabriel, 2003; Yu & McVey, 2010). An important role of BLM is to inhibit CtIP/MRE11 (Grabarz *et al.*, 2013). MMEJ occurs during G2-S or G1 (Truong *et al.*, 2013). It processes ssDNA gaps of 2 to 25 nucleotides in length (McVey & Lee, 2008), while ssDNA gaps >30 nucleotides are repaired by ssDNA annealing (SSA). Proteins involved in MMEJ include BLM/MRN, EXO1 or DNA2, FEN1, DNA polymerase β, λ or μ, Ligase I or Ligase III/XRCC1 and MMR proteins (Figure 1) (Crespan *et al.*, 2012; Lee-Theilen *et al.*, 2011; Nimonkar *et al.*, 2011; Paul *et al.*, 2013). After ssDNA is annealed in the gap region, mismatched bases are corrected by MMR. DNA polymerase β, λ or μ fills gaps and FEN1 processes the overhangs.

53BP1 and γH2AX are DNA damage sensors that also play a major role in class switch recombination (CSR). Lymphocytes depleted of 53BP1 or γH2AX display impaired CSR, due to the lack of protection of broken DNA ends, which results in enhanced resection-associated alternative end joining (MMEJ) and inhibition of C-NHEJ repair (Bothmer *et al.*, 2013). It has been reported that phosphorylated 53BP1 and BLM interact directly during DSB repair (Sengupta *et al.*, 2004; Tripathi *et al.*, 2008). Moreover, 53BP1 and TopIIIα have been shown to regulate BLM in cell cycle-dependent manner (Grabarz *et al.*, 2013). In cells absent of 53BP1 or RIF, BLM promotes long deleterious resection mediated by CtIP/MRN (Grabarz *et al.*, 2013). It has been proposed that RECQL5β can substitute for BLM in DSB repair (Branzei & Foiani, 2007), and that RECQL1 or RECQL5β can substitute for BLM to suppress SCE. Mouse ES cells with knockout of one allele of *Blm* and *Recql5* had a higher rate of SCE after UV-induced DNA damage than cells with knockout of both alleles of *Blm* or *Recql5* (i.e. single gene KO) (Hu *et al.*, 2005). *Recql5*-deficient mouse ES and MEF cells show a profound reduction in DNA replication after the treatment with, topoisomerase I inhibitor, camptothecin (Hu *et al.*, 2009). However, BLM and RECQL5β do not interact directly, and there is no evidence to date that RECQL5β plays a role in B-NHEJ

or MMEJ. For a more extensive discussion of the role of RECQL5β in HR, see Popuri *et al.* (2013).

Class switch recombination

Immature B-cells express B-cell receptors (BCR) on the surface and undergo maturation via class switch recombination (CSR) after migration from the bone marrow to spleen and lymph nodes. During BCR maturation, targeted recombination is carried out in the switch (S) region of immunoglobulin heavy chain genes. CSR takes place in B-cells during G1 and is initiated by activation-induced deaminase (AID). AID deaminates cytosine to uracil in the switch region, leading to a mutagenic process mediated by uracil-DNA glycosylase, and enzymes of MMR, C-NHEJ and/or MMEJ (reviewed in Kotnis *et al.* 2009). *Xrcc4*$^{-/-}$ mice show increased resection at DNA termini in the cleaved S region, which can promote oncogenic translocations (Boboila *et al.*, 2010; Simsek & Jasin, 2010), but CSR still occurs. A recent study by Bothmer and co-workers (2013) investigated the contribution of WRN and BLM to CSR. Their results suggest that resection depends on both CtIP and EXO1. Additionally, inhibition of CtIP partially rescues the CSR defect in 53BP1-and H2AX-deficient lymphocytes as does interference with the RecQ helicases BLM and WRN. They concluded that BLM and WRN may contribute to the repair of AID-mediated DNA lesions that occur within the repetitive G-rich and highly transcribed switch regions in B lymphocytes, and that minimizing resection favors efficient CSR (Bothmer *et al.*, 2013; Stavnezer *et al.*, 2008). In addition, Babbe and colleagues (2009) showed that BLM-deficient lymphocytes show altered CSR in Iga1, Iga2 and Iga3 genes in splenic B cells, and BLM-deficient B cells have a mild shift towards MMEJ. Additionally, they found that p53-deficient conditional *Blm* mice showed an increase in propensity for B cell lymphoma development, and that the cells from these mice showed impaired cell cycle progression and survival and high rates of chromosomal structure abnormalities. Their data suggests that BLM and p53 cooperate in avoiding lymphoma development and in maintaining chromosomal stability (Babbe *et al.*, 2009).

C-NHEJ in human disease

Mice depleted in *Ku70*, *Ku80*, *Xrcc4* or *DNA-PKcs* show premature aging. The *Ku70*$^{-/-}$ and *Ku80*$^{-/-}$ mice are defective in NHEJ, and display premature aging characterized by osteoporosis, growth failure, incomplete plate closure, atopic skin disease, liver pathology, sepsis, cancer and short life span (Gu *et al*., 1997; Nussenzweig *et al*., 1996, 1997). In *Ku70*$^{-/-}$ and *Ku80*$^{-/-}$ mice, V(D)J rearrangement is defective (Gu *et al.*, 1997; Ouyang *et al.*, 1997). In humans, a mutant allele of DNA-PKcs causes severe combined immune disease (SCID), by way of its inability to activate Artemis endonuclease (van der Burg *et al*., 2009). Recent reports suggest that impairment of Ku70 in DNA repair is associated with Huntington's disease (Enokido *et al.*, 2010; Tamura *et al.*, 2011). In humans, no disease has been directly linked to defects in Ku70 or Ku80.

Telomere maintenance

DNA repair enzymes involved in telomere maintenance include Ku70/80, DNA-PKcs, MRN, BLM, WRN and RECQL4. When telomere length or structure is improperly

maintained, uncapped telomeres are recognized by DNA damage response proteins, such as 53BP1 and γ-H2AX. If telomeres are not restored, genetic rearrangements and mutations can accumulate. Cancerous cells, which are deficient in telomere shortening, have activated telomerase or activate the pathway of alternative lengthening of telomeres (ALT) resulting in the so called ALT cells (Bechter *et al.*, 2004; Blasco, 2005). Cells that are immortalized by Simian Virus 40 (SV40) or Epstein Barr Virus (EBV) infection activate the ALT pathway or disregulate the shelterin complex, leading in both cases to the formation of ALT cells (Bechter *et al.*, 2004; Kamranvar *et al.*, 2013). The frequency of telomere fusion is higher in ALT cells than in control cells, and it has been suggested that Ku70/80 plays a major role in telomere fusion in ALT cells (Espejel *et al.*, 2002). However, telomere fusion also occurs in *Ku70*$^{-/-}$*/80*$^{-/-}$ MEFs depleted in shelterin proteins TRF1/2 (Rai *et al.*, 2010), suggesting that telomere fusion is supported by an alternative end joining pathway (Indiviglio & Bertuch, 2009; Sfeir & de Lange, 2012). PARP1 promotes B-NHEJ. This is evidenced by the fact that *Parp1*$^{-/-}$ MEFs show an increase in chromosome end-to-end fusions or chromosome ends without detectable telomeric DNA after induction of DNA damage (Gomez *et al.*, 2006). PARP1 associates with TRF2, and is capable of poly(ADP-ribosyl)ation of TRF2, which affects its binding to telomeric DNA (Gomez *et al.*, 2006). This demonstrates that neither C- nor B-NHEJ, but MMEJ has a role in the telomeric fusion (Sfeir & de Lange, 2012). Furthermore, *53bp1*$^{-/-}$ MEFs depleted in TRF1/2 or Ku70/80 show increased resection of 5′ telomere ends, likely mediated by CtIP/BLM/EXO1 (Sfeir & de Lange, 2012). One interpretation of these data is that the shelterin complex plays a primary role in protecting 5′ telomeric ends, while Ku70/80 and 53BP1 play a secondary role by suppressing B-NHEJ (Rai *et al.*, 2010; Sfeir & de Lange, 2012).

In ALT cells, WRN and BLM helicases have clear roles in telomere maintenance. WRN unwinds G-quaduplex DNA during telomere replication, and facilitates formation and resolution of T-loops (Paeschke *et al.*, 2010). WRN also promotes repair of oxidative lesions in D-loops (Ghosh *et al.*, 2009). In *WRN*$^{-/-}$ ALT cells, BLM suppresses SCE in telomeric DNA, which suggests that BLM does not need WRN to suppress SCE (Mendez-Bermudez *et al.*, 2012). Recently, it was shown that ALT cell induction is positively regulated by the proteins RAD17 and BLM, and negatively regulated by EXO1 and DNA2 (O'Sullivan *et al.*, 2014). DNA-PKcs cooperates with WRN in D-loops and prevents shortening of the telomeric G-strands *in vivo* (Kusumoto-Matsuo *et al.*, 2010). Cells from RTS patients accumulate DNA fragile sites in telomeres (Ghosh *et al.*, 2012), suggesting a significant role for RECQL4. In telomere maintenance, WRN and RECQL4 may both have specialized roles during telomere replication (Ghosh *et al.*, 2012). *In vitro* assays suggest that RECQL4 may be stimulated by either TRF1/2 or by WRN, and this may play a major role during unwinding of D-loops during telomere replication (Ghosh *et al.*, 2012).

Conclusion and perspectives

This review summarizes the roles of human RecQ helicases in C-NHEJ, B-NHEJ and other important DNA end-joining processes in human cells (Figure 2). The data from several publications demonstrate that RecQ helicases are dynamic, multifunctional proteins, playing numerous roles in DNA metabolism. There is significant crosstalk among the pathways, as RecQ helicases cooperate and/or complement each other during DNA end-joining. MMEJ is

less well characterized than other DNA end-joining pathways; however, the role of BLM in stimulating the exonuclease EXO1 and DNA2 during MMEJ is well-documented. The roles for human RecQ helicases in CSR are not yet well understood. Future studies should focus on RECQL1, RECQL4 and RECQL5β, whose biological roles are less well characterized than BLM and WRN. While not the subject of this review, RECQL4 has been implicated in BER (Kumata *et al.*, 2007; Schurman *et al.*, 2009), and RECQL5β may play a major role in HR-dependent DSB repair, especially after replication fork collapse (Popuri *et al.*, 2012b, 2013; Schwendener *et al.*, 2010).

Given the critical roles of RecQ helicases in the response to genotoxic stress, it appears that they may be master regulators of the response to genomic instability. The abundant literature in this regard highlights the importance of DNA damage and DNA repair in aging. A corollary of this fact is that DNA repair enzymes, including RecQ helicases, prevent aging-associated disease at all life stages.

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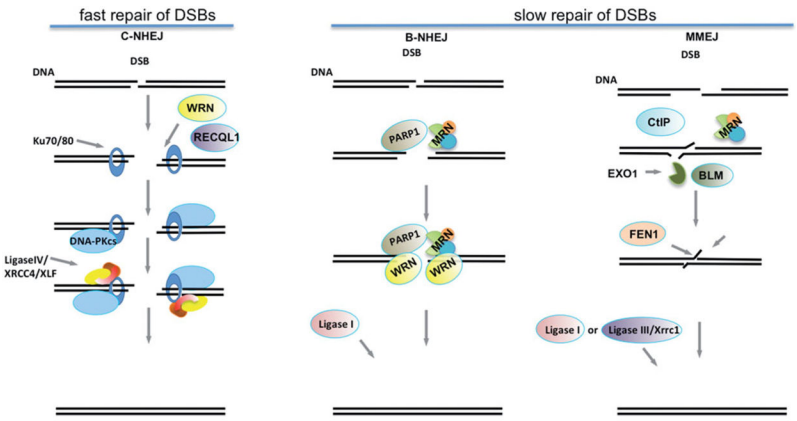

Figure 1.

Schematic diagram of DNA end-joining pathways. C-NHEJ, canonical non-homologous end-joining; B-NHEJ, alternative non-homologous end-joining; MMEJ, micro-mediated end-joining; MRN, MRE11-RAD50-NBS1 complex. EXO1/BLM/MRN or BLM/DNA2/MRN carry out CtIP-stimulated end-resection during MMEJ. (see colour version of this figure at www.informahealthcare.com/bmg).

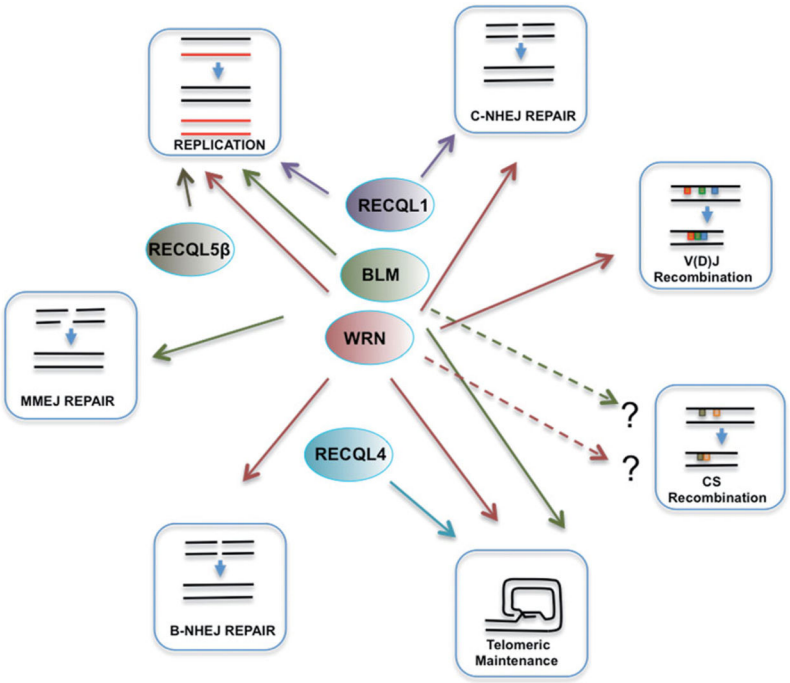

Figure 2.

BLM, WRN, RECQL4, RECQL1 and RECQL5β in DNA end-joining, DNA homologous recombination and DNA replication. Only BLM is known to play a major role in MMEJ, SSA and CSR. (see colour version of this figure at www.informahealthcare.com/bmg).