



HHS Public Access

Author manuscript

Annu Rev Biochem. Author manuscript; available in PMC 2015 September 29.

Published in final edited form as:

Annu Rev Biochem. 2014 ; 83: 519–552. doi:10.1146/annurev-biochem-060713-035428.

Human RecQ Helicases in DNA Repair, Recombination, and Replication

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Abstract

RecQ helicases are an important family of genome surveillance proteins conserved from bacteria to humans. Each of the five human RecQ helicases plays critical roles in genome maintenance and stability, and the RecQ protein family members are often referred to as guardians of the genome. The importance of these proteins in cellular homeostasis is underscored by the fact that defects in BLM, WRN, and RECQL4 are linked to distinct heritable human disease syndromes. Each human RecQ helicase has a unique set of protein-interacting partners, and these interactions dictate its specialized functions in genome maintenance, including DNA repair, recombination, replication, and transcription. Human RecQ helicases also interact with each other, and these interactions have significant impact on enzyme function. Future research goals in this field include a better understanding of the division of labor among the human RecQ helicases and learning how human RecQ helicases collaborate and cooperate to enhance genome stability.

Keywords

RECQL1; BLM; WRN; RECQL4; RECQL5; genome stability

INTRODUCTION

Helicases are ubiquitous enzymes that play critical roles in DNA replication, recombination, repair, and transcription. A recent genome-wide analysis predicted 95 genes encoding distinct helicases in the human genome (1). Most mammals possess five RecQ helicases: RECQL1, BLM, WRN, RECQL4, and RECQL5. Defects in human BLM, WRN, or RECQL4 cause monogenic disease syndromes; all RecQ helicases play critical roles in genome maintenance and are often referred to as guardians of the genome (2, 3). Although bacteria and yeast were initially thought to express only one RecQ helicase, recent studies suggest that additional homologs are present (4–7).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Most members of the DNA helicase superfamily use energy derived from ATP hydrolysis to unwind double-stranded DNA (dsDNA), including complex DNA secondary structures, and many helicases also remodel protein–DNA complexes. RecQ helicases bind to and translocate along single-stranded DNA (ssDNA) in a 3′ → 5′ direction, and ssDNA stimulates their dsDNA-unwinding activity. RecQ helicases are structure-specific DNA-binding proteins. Some promote branch migration or disrupt Holliday junctions (HJs), and all human RecQ helicases catalyze ssDNA annealing [recently reviewed by Wu (8)].

RecQ helicases share three highly conserved protein domains (9), the core helicase domain (composed of HD1 and HD2), the RecQ C-terminal (RQC) domain, and the helicase and RNase D–like C-terminal (HRDC) domain (Figure 1*a, b*). In addition, they possess unique protein regions and motifs that direct subcellular localization (Figure 1*a*). Previously, it was thought that RECQL4 lacked the RQC domain, but a recent bioinformatics analysis suggests that it may have an RQC domain (10). RECQL5 lacks part of the RQC domain and, similar to RECQL1 and RECQL4, lacks the HRDC domain (11). Unique protein segments confer distinctive functions or properties to each homolog, including specific protein–protein interactions, oligomerization signals, and nuclear or mitochondrial localization (Figures 1*a* and 2).

This article presents a comprehensive review and discussion of recent literature on human RecQ helicases, emphasizing advances toward understanding the RecQ helicase mechanisms of action and the unique and/or redundant roles each human RecQ helicase plays in DNA repair, recombination, and replication. As discussed below, critical unresolved questions in the field are whether and how the unique and overlapping roles of the five human RecQ helicases are coordinated in DNA metabolism.

CONSERVED RecQ HELICASE DOMAINS

Core Helicase Domain

There are six distinct helicase protein superfamilies (SF1–6), and their members share conserved sequence motifs as well as structural and mechanistic features (12, 13). Human RecQ helicases belong to the SF2 helicase superfamily (see the conserved motifs in the helicase core domains in Figure 1*b*). The RecQ proteins display strong correlations between the conserved motifs and functional DNA helicase activity, such that they unwind a wide variety of DNA substrates, many of which resemble DNA repair intermediates. Table 1 summarizes the substrate specificities of *Escherichia coli* and human RecQ helicases.

RecQ C-Terminal Domain

In most cases, the minimal helicase functional unit includes the helicase and RQC domains (14–16). The RQC structure was solved by X-ray crystallography; it features a zinc-binding motif, a helix-hairpin-helix, winged-helix (WH) domain, and a β -hairpin motif (17–19). The RQC domain specifically mediates binding to G quadruplex (G4) DNA (20) and stabilizes binding to other DNA structures. The purified WH domain from WRN (15, 19, 21, 22) and BLM (20) shows strong DNA-binding activity. The crystal structure of the RQC domain of WRN [Protein Data Bank (PDB) identifier 3AAF] shows that the WH domain makes contacts with the phosphate backbone on the 5′ ssDNA overhang (19). Consistent with these

results, mutagenesis of conserved Arg987 and Arg993 in the WH of WRN inactivates DNA-binding and helicase activity (19, 23). In the RECQL1–DNA cocrystal (PDB 2WWY) (24), the WH domain binds dsDNA, and the β -hairpin motif binds near the junction between dsDNA and ssDNA (Figure 1c). The β -hairpin motif in the RQC domain of RECQL1 regulates dimer formation and couples ATPase activity to DNA unwinding (18, 25). Notably, oligomerization of RECQL1 regulates the opposing catalytic activities of helicase and strand annealing (SA) (see the sidebar titled Structure and Oligomeric Status of RecQ Helicases). Thus, the RQC domain directs binding to ssDNA–dsDNA junctions, and the β -hairpin couples ATP hydrolysis to DNA unwinding.

STRUCTURE AND OLIGOMERIC STATUS OF RecQ HELICASES

The structure and oligomeric status of the RecQ helicases are exciting areas of analysis. RecQ helicases are ssDNA-stimulated ATPases; however, the mechanism for the stimulation is not well understood. These proteins contain a highly conserved sequence, the aromatic loop (24, 295), immediately following the Walker B motif (important for ATP hydrolysis), which is a true signature sequence for the family. The cocrystal structure shows that the aromatic loop lies in close proximity to DNA (Figure 1c), and mutagenesis of the loop in *E. coli* or human RECQL1 suggests this sequence regulates dimerization and that it may be the DNA-binding sensor that couples ATPase to DNA binding (25, 295). In general, helicase subunit oligomerization is another unanswered fundamental question. Interestingly, BLM and WRN form hexamers and trimers (57, 296), and RECQL1 monomers and dimers have been observed (reviewed in Reference 297). RECQL4 exists primarily as a dimer (298), and RECQL5 is a monomer (11). Recent studies suggest that the oligomeric status regulates the catalytic activities of RECQL1, allowing it to toggle between DNA unwinding, DNA strand annealing, and HJ branch migration/disruption (299). Thus, oligomerization represents a mechanism whereby the helicases could potentially be differentially regulated.

Helicase and RNase D–Like C-Terminal Domain

The HRDC domain is found in WRN and BLM but not in other human RecQ enzymes, suggesting that it plays a specialized role. Consistent with this finding, the HRDC domain in yeast Sgs1 and *E. coli* RecQ promotes stable DNA binding (26, 27) but is dispensable for ATPase and helicase activities (27, 28). In WRN and BLM, the HRDC domain plays a role in recruitment to laser-induced dsDNA breaks (DSBs) (29, 30), and methyl methanesulfonate– and mitomycin C–induced DNA damage (31). Thus, the HRDC domain promotes the localization of WRN and BLM to specific DNA lesions.

THE RecQ PROTEIN FAMILY

RecQ homologs interact with a wide variety of protein partners and play multiple important roles in DNA metabolism (Figure 2). Some partners are unique, but many are common to more than one RecQ protein. Defects in human BLM, WRN, and RECQL4 cause rare recessive autosomal diseases for which there are currently no effective therapies. Mutations in *BLM* (15q26.1) cause Bloom syndrome (BS) [Online Mendelian Inheritance in Man

(OMIM) code number 604610]; mutations in *WRN* (8p12) cause Werner syndrome (WS) (OMIM 604611); and mutations in *RECQL4* (8q24.3) cause Rothmund–Thomson syndrome (RTS) (OMIM 268400), RA-PADILINO syndrome (OMIM 266280), and Baller–Gerold syndrome (BGS) (OMIM 218600). Each syndrome has unique clinical and developmental patterns, suggesting that each human RecQ helicase has some specialized biological function, even though all five human RecQ helicases are thought to widely contribute to genomic stability.

RECQL1

RECQL1 is the smallest (72 kDa) and most abundant human RecQ helicase (recently reviewed in Reference 36). Immunohistochemical studies show that human RECQL1 is widely expressed in the nucleus (32) and that messenger RNA encoding murine *Recql1* is ubiquitous, with highest expression in testis (33). Mice lacking *Recql1* are phenotypically normal; however, mouse embryonic fibroblasts demonstrate chromosome instability, including aneuploidy, spontaneous chromosomal breakage, frequent translocations, hypersensitivity to ionizing radiation, DNA damage, and elevated spontaneous sister chromatid exchanges (SCEs) (33). Many diverse studies show that defects in or depletion of RECQL1 impairs cell growth, causes genome instability, or induces mitotic catastrophe (33–37). RECQL1 is proposed to have a unique role during replication origin firing and nascent DNA synthesis (38), and it is critical for replication fork restart (39). RECQL1 is highly expressed in a variety of human solid tumors (40), but there are no known diseases associated with defects in RECQL1.

BLM

Expression of BLM increases in the S and G₂/M phases (41), and it is localized to promyelocytic leukemia bodies and telomeres in the nucleus (42, 43). BLM is rapidly recruited to damaged DNA (42, 44). It is an essential gene in the mouse (45–47), given that defects in or depletion of murine *Blm* is linked to an increase in apoptosis during embryogenesis and to severe anemia, chromosomal instability, and a high incidence of cancer in adult mice (45, 46, 48, 49). Cell lines derived from *Blm*-defective mice and patient-derived human BS cells demonstrate a hyperrecombination phenotype.

The most prominent clinical feature of BS is dwarfism, which is due to pre- and postnatal growth retardation (Supplemental Table 1; follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>) (50). BS patients prematurely develop cancer, diabetes, and chronic progressive lung disease. The types and frequencies of cancers observed in BS patients are similar to those in the general population; however, BS patients develop cancer early, and they develop multiple primary tumors (51). Cancer is the leading cause of death, often in childhood, among BS patients (51).

WRN

In undamaged human cells, WRN localizes predominantly to the nucleolus, but after DNA damage, it rapidly mobilizes to other nuclear regions (29, 52–54). Immunohistochemical staining of S-phase cells reveals a punctate pan-nuclear distribution of WRN, suggesting that WRN may be associated with nascent replicating DNA and telomeres (55).

WRN is unique among RecQ helicases in possessing an N-terminal 3' → 5' exonuclease (56), similar to *E. coli* DnaQ-like replication-associated bacterial exonucleases (57). Forked dsDNA and bubble structures with 3'-recessed ends are the preferred DNA substrates of the WRN exonuclease (58–60), which is unable to bypass certain oxidative and bulky DNA lesions (61–65). Although the in vivo role of the WRN exonuclease in DNA metabolism is not yet known, it is possible that the WRN exonuclease proofreads repair tracts synthesized by translesion DNA polymerases (see the section titled RecQ Helicases in DNA Replication). The collaboration between the WRN exonuclease and helicase is another important area for future research.

Wrn-knockout mice have no overt pathology and fail to recapitulate features of clinical WS (66, 67). However, other *Wrn*-null mice are insulin resistant and prone to diabetes and obesity when fed a high-fat and sugary diet (68), and telomere shortening and premature aging are observed in double-mutant mice with null alleles of *Wrn* and *Terc*, the RNA components of telomerase (69). The latter result suggests that long telomeres in wild-type mice might mask the effect of the *Wrn*-null mutation on genome stability.

Recently, the clinical features of WS patients were reviewed in detail and therefore are not discussed in detail here, except to note that cancer and atherosclerosis are the leading causes of death, with susceptibility to specific types of rare cancer as well as multiple primary or sequential neoplasms (70, 71). The clinical features of BS, WS, and RECQL4 syndrome are summarized in Supplemental Table 1.

RECQL4

RECQL4 partitions between the nucleus and cytosol in a manner that varies in different cell types and tissues (72). RECQL4, WRN, and BLM are thought to play roles in telomeric DNA maintenance, but RECQL4 is unique in localizing to mitochondria (73–75). RECQL4 expression peaks during S phase and is highest in the thymus and testis (41).

Three mouse models for *Recql4* have been characterized in which specific exons were deleted as follows: Δ exons 5–8, Δ exons 9–13 (the entire helicase domain), and Δ exon 13 (the last exon in the helicase domain). Mice with deleted Δ exons 5–8 die as embryos (76); 95% of the mice with deleted Δ exon 13 die perinatally (77), and the “escapers” fail to thrive. Mice with deleted Δ exons 9–13 have palate and limb defects, as well as poikiloderma-like features, and are susceptible to cancer in the absence of *APC* (the tumor-suppressor adenomatous polyposis coli gene) (78). *Recql4*-deficient mice lacking Δ exons 9–13 have a normal life span.

Mutations in *RECQL4* cause type II RTS (79), a disease characterized by hypo- and hyperpigmentation, punctate atrophy, and telangiectasia (three symptoms known collectively as poikiloderma), as well as congenital skeletal abnormalities (reviewed in Reference 80). The leading cause of death for patients with RECQL4 mutations is cancer (L.L. Wang, personal communication), whereas life expectancy in cancer-free RTS patients is normal (80). The characteristic clinical feature of BGS is congenital coronal craniosynostosis (81). Poikiloderma is not observed in *RECQL4*-defective patients with RAPADILINO syndrome, but these patients are susceptible to rare cancers (82). RTS is

more prevalent than BGS or RAPADILINO syndrome. See Supplemental Table 1 for a comparison between the RECQL4-related disorders.

Mutations causing RTS, BGS, and RAPADILINO syndrome are found in all RECQL4 protein domains. However, of the missense mutations available for analysis, those that compromise RECQL4 structurally segregate with RTS, whereas those that impair helicase activity segregate with RAPADILINO syndrome (83, 84). Additional mutational analysis of RECQL4 is warranted. For further information about RECQL4's protein interaction partners, see Figure 2 and Reference 85.

RECQL5

There are three alternatively spliced forms of human RECQL5: RECQL5 α , RECQL5 β , and RECQL5 γ (86). RECQL5 β (referred to here as RECQL5) is the most abundant isoform. RECQL5 is ubiquitously expressed in a cell-cycle phase- and cell type-independent manner, contains a nuclear localization signal, and is an ATP-dependent DNA helicase (Figure 1a) (11, 41, 86, 87). RECQL5's protein interaction partners were described in a recent review (88), and some are shown in Figure 2. Most notably, RECQL5 binds both initiating and elongating forms of RNA polymerase II and inhibits transcription (89, 90).

RQC-5-deficient *Caenorhabditis elegans* develop normally but show a 37% reduction in life span and increased sensitivity to ionizing radiation (91). *Recql5*-knockout mice also develop normally but display cancer susceptibility (92). Cancer-free *Recql5*-deficient mice have a normal life span. *Recql5*-deficient mouse embryonic fibroblasts accumulate endogenous DNA damage, possibly due to faulty DSB repair by homologous recombination (HR), and display dramatically increased frequencies of gross chromosomal aberrations after exposure to camptothecin (CPT). Thus, model organisms lacking RECQL5 confirm that RECQL5 is not essential for embryonic development but demonstrate that it is important for tumor suppression, and suggest that the functions of RECQL5 are evolutionarily conserved.

RecQ HELICASES IN DNA REPAIR

RecQ helicases demonstrate specific and preferred binding to DNA substrates that resemble DNA repair intermediates (Table 1), suggesting that they play a role in DNA repair. Consistent with this finding, RecQ helicases interact with and modulate the activity of proteins that play critical roles in DNA repair. Studies in mouse models confirm this putative role and suggest additional roles in recombinational DNA repair and DNA replication. RecQ helicases are currently thought to influence base excision repair (BER) directly and indirectly play a significant role in DSB repair, but have a less clear influence on nucleotide excision repair (NER) and mismatch repair. These roles are discussed at length below, with an emphasis on how each RecQ impinges on distinct DNA repair pathways in unique and/or overlapping ways. Figure 3 summarizes this discussion.

Base Excision Repair

BER removes oxidative, abasic, and other monofunctional base modifications from DNA. Base lesions are recognized by a glycosylase that removes the damaged nitrogenous base while leaving the sugar-phosphate backbone intact, creating an apurinic/apyrimidinic (AP)

site. Mammalian cells express multiple DNA glycosylases with overlapping DNA lesion specificity. AP endonuclease 1 (APE1) is the next enzyme in the BER pathway; it cleaves AP sites, producing a single-strand break intermediate, which can be utilized by DNA polymerase β (Pol β) for DNA repair synthesis, and is followed by ligation of nicked DNA by a DNA ligase (93).

WRN, but not other human RecQ helicases, strongly stimulates NEIL1 glycosylase (94), which incises formamidopyrimidines, 5-hydroxyuracil, and to a lesser extent, 8-oxoG. APE1 is inhibited by WRN (95) but stimulated by RECQL4 (96) in vitro. APE1 is upregulated in RTS cells (96) and is overexpressed in some sarcomas, cancers that are common to RTS and WS (97). Pol β is significantly stimulated by helicase-proficient, but not by helicase-defective, WRN (98). Pol β strand displacement synthesis is stimulated by RECQL4 (96) but not by RECQL5 (99). DNA Pol β lacks 3' proofreading exonuclease activity, so when DNA Pol β and WRN work in a coordinated manner, WRN exonuclease activity can play a role in correcting DNA synthesis errors, contribute to postreplication repair, and increase DNA synthesis fidelity during BER (100).

WRN physically and functionally interacts with proteins involved in short-patch BER, in which one base is replaced, and long-patch BER, in which up to eight bases are replaced (Figure 3a). The replicative protein flap endonuclease 1 (FEN1) plays a role in long-patch BER and interacts with most of the human RecQs (Figure 2) (96, 99, 101–103). Cooperation between FEN1 and the RecQ helicases, and/or stimulation of FEN1 by one of the enzymes, may also promote postreplication DNA repair (104).

WRN, RECQL1, and RECQL4 interact with and are modulated by poly(ADP-ribose) polymerase 1 (PARP1). PARP1 adds poly(ADP-ribose) moieties to chromatin-binding proteins, thereby modulating chromatin structure and function (105, 106). WRN-deficient cells fail to activate PARP1 in response to oxidative and alkylation DNA damage, indicating that the WRN–PARP1 interaction is biologically relevant and significant (107). However, PARP1 is hyperactivated in RECQL1-depleted cells exposed to oxidative stress and in BLM-, WRN-, and RECQL5-depleted cells that are not exposed to exogenous stress (108–110). Interestingly, WRN and RECQL1 bind to and are inhibited by poly(ADP-ribose) (39, 108, 111). The C-terminal region of RECQL4 is a PARP1 substrate, and PARP1 inhibitors alter the cellular localization of RECQL4 (112). The complex interactions between PARP1 and RecQ helicases warrant further study, especially in light of the critical role played by PARP1 in cancer susceptibility and recent reports of PARP1 as a cancer chemotherapeutic target (113).

RECQL5, but not the other RecQ helicases, is recruited to laser-induced ssDNA breaks in vivo, and RECQL5-depleted cells are sensitive to and accumulate oxidative DNA damage (110). RECQL5 does not stimulate APE1 or Pol β (99), but it may positively regulate transcription of *PARP1* and *XRCC1* (110). Thus, RecQ helicases may modulate BER indirectly by regulating transcription of BER-related genes (96, 110).

Nucleotide Excision Repair

NER removes bulky lesions from DNA, such as UV-induced pyrimidine dimers and carcinogen adducts (for a review, see Reference 114). Limited evidence suggests that RecQ helicases might play a role in NER. For example, the NER protein XPG (xeroderma pigmentosum complementation group G) stimulates WRN helicase (115), and the XPA protein interacts with RECQL4 (116). However, RecQ helicase-deficient cells are not hypersensitive to UV irradiation, and there is no direct evidence that the RecQ helicases alter NER functionally in vivo. Thus, the significance of the observed interactions remains unclear.

DNA Double-Strand Break Repair

DNA DSBs can cause transient or permanent cell-cycle arrest, mutagenesis, gross chromosomal rearrangements, and ultimately cell death or tumorigenesis. Human cells can repair DSBs by nonhomologous end joining (NHEJ), alternative nonhomologous end joining (Alt-NHEJ), or HR (Figure 3b) (117, 118). HR-dependent DSB repair is based on a sister chromatid template, is error free, and occurs only during the late S and G₂ phases (119). NHEJ and Alt-NHEJ occur throughout the cell cycle and are error prone (reviewed in Reference 117). Alt-NHEJ is active mainly when NHEJ is impaired (117). As described below, all five human RecQ helicases play putative roles in one or more subpathways of DSB repair.

Nonhomologous end joining—Nonproliferating human cells repair DSBs by NHEJ in a reaction that requires 53BP1, the Ku70/Ku80 heterodimer (Ku), DNA-PKcs, and XLF/XRCC4/LIG4 (120, 121). When necessary, nonligatable ends are processed in an intermediate step by Artemis or a DNA polymerase-associated exonuclease. 53BP1 promotes NHEJ by suppressing DSB 5'-end resection (122, 123). Figures 2 and 3b summarize the interactions between RecQ helicases and NHEJ proteins. For example, WRN interacts with Ku and is a substrate of the DNA-PKcs kinase (124–127). Ku has no effect on WRN helicase or ATPase activities but strongly stimulates the WRN exonuclease (124). In contrast, DNA-PKcs stimulates the WRN helicase but not the WRN exonuclease (128); however, the WRN exonuclease is also stimulated by XRCC4/LIG4 (129). WS cells are mildly sensitive to ionizing radiation-induced DSBs (126), but in the context of in vitro DSB repair, they are competent in ligating linear plasmid DNA (130). However, WS cells exhibit elevated frequencies of deletions in chromosomal DNA (131, 132), and likewise, more deletion mutations are recovered from in vitro plasmid ligation assays (57, 133).

RECQL1 may also play a role in NHEJ (134) because *Recql1*-deficient mice (33) and human cells (34) are defective in DSB repair. RECQL1 interacts with Ku, and extracts from RECQL1-null cells display wild-type levels of plasmid end-joining activity and reduced Ku-DNA-binding activity (134). Whether WRN and RECQL1 play compensatory or redundant roles in NHEJ at the Ku step is unknown.

Alternative nonhomologous end joining—The precise mechanism of Alt-NHEJ is poorly understood; however, there is often microhomology between the DSB break point and the DNA repair template (117). PARP1 may act as a DNA damage recognition protein

during Alt-NHEJ (135, 136), followed by end resection by MRE11, CtIP, and EXO1 (137–140) and ligation by LigIII/XRCC1 (135, 136, 141). WRN, BLM, and RECQL1 interact with EXO1 (136, 142–145) and MRE11/RAD50/NBS1 (MRN) (146–148). WRN also interacts with LigIII α (149). Although preliminary, these findings are consistent with the possibility that RecQ helicases modulate Alt-NHEJ.

When 53BP1-defective human cells were treated with a small-molecule WRN inhibitor or a hypomorphic *Blm* allele (*Blm*^{3/3}) was expressed, an I-SceI intrachromosomal break was repaired normally by Alt-NHEJ, but the efficiency of B cell class-switch recombination, which requires Alt-NHEJ, increased (140, 150). To explain these results, the investigators proposed that 53BP1 and phosphorylated histone H2AX (γ -H2AX) compete with proresection enzymes, WRN and BLM, for DSBs and that the circumstances that limit DNA resection in B cells promote Alt-NHEJ and class-switch recombination (140).

Homologous recombination—dependent double-strand break repair—HR is a conserved cellular process that occurs in the late S and G₂ phases of the cell cycle. It repairs DNA DSBs when a homologous template is available. HR is involved in DSB repair at stalled replication forks during S phase, promotes repair at DNA lesions during mitosis, and can promote correct chromosomal pairing or exchange during meiosis. Some of the proteins required for HR-mediated DSB repair include CtIP, MRN, RPA, DNA2, EXO1, and RAD51 (118). Notably, RPA, a human ssDNA binding protein that is essential for DNA replication and repair, interacts with all human RecQ proteins and stimulates their helicase functions (Figures 2 and 3*b*) (11, 151–154).

DNA end resection is an exonucleolytic process that generates ssDNA protruding tails at a DNA DSB. In human cells, the first step toward resection is the binding of CtIP and MRN at the DSB (148, 155). CtIP stimulates the MRN exonuclease, and MRN recruits two additional nucleases, EXO1 and DNA2. RPA also plays a role by stimulating DNA unwinding using BLM and enforcing 5′–3′ resection polarity by DNA2 (148). Alternatively, EXO1, instead of MRN, can catalyze excision at the DSB. BLM increases the affinity of EXO1 for DNA ends independently of its helicase, whereas MRN recruits and enhances the processivity of EXO1 (148). In vitro, only BLM, but not WRN, RECQL4, or RECQL5, stimulates DNA end resection by DNA2 (148); however, RECQL1, BLM, and WRN stimulate EXO1 (142, 144, 145). Even though WRN has inherent exonuclease activity, the 3′–5′ polarity is counterproductive and thus would inhibit recombination. Therefore, it appears that BLM, via its interactions with both EXO1 and DNA2, plays a dominant role in promoting DSB resection.

In yeast, these complexes are conserved because Sgs1 (RecQ homolog in *Saccharomyces cerevisiae*), Dna2, and RPA (156), or Sgs1, Exo1, and Sae2, catalyze DNA end resection (143, 157, 158). Interestingly, in *Xenopus*, xWRN, xDNA2, and RPA are implicated in DNA end resection (159, 160).

Evidence suggests that Sgs1, BLM, and RECQL5 act as antirecombination factors because they inhibit an early step in HR-mediated DSB repair. They do so by disrupting the RAD51 nucleoprotein filament, which is essential for the homology search and D-loop formation

(92, 161–163). WRN, RECQL1, and RECQL4 also colocalize with RAD51; however, WRN and RECQL1 cannot disrupt RAD51 filaments (92, 162), whereas RECQL4 has not been tested. Thus, BLM and RECQL5 may be partially complementary to one another at this step. An analysis of SCEs in BLM and RECQL5 single- and double-deficient chicken and mouse cells supports the idea that RECQL5 may play additional roles in suppressing SCEs when BLM function is impaired (164, 165).

Although WRN does not have the same antirecombinogenic activity as BLM, it plays a role in DSB pathway choice and suppresses recombination (166, 167). Ectopic expression of an HJ resolvase in WS cells enhances viability and proliferation, suggesting that there is defective HR in these cells (168, 169). How WRN facilitates this action is unclear, but it interacts with several HR proteins, including MRN (146), BRCA1 (170), RAD52 (53), RAD51, and RAD54 (Figure 2) (171).

RECQL4 is also recruited to laser-induced DSBs, and RECQL4-deficient cells are moderately sensitive to ionizing radiation (54, 172). RECQL4 interacts with RAD51 in cells exposed to etoposide (173), an inhibitor of topo-isomerase II (Topo II) that generates DSBs. Although these are preliminary findings, the data support a role for RECQL4 in DSB repair.

Homology-dependent branch migration and DNA synthesis–dependent strand annealing (SDSA) generate HJs or double HJs (dHJs), and RecQ helicases (RECQL1/WRN/BLM) interact with and can branch migrate HJs and dHJs or dissolve them (BLM/Topo/RMI1/RMI2), as discussed in more detail below (174–179). Alternatively, HJs can be resolved endonucleolytically by nucleases (GEN1, MUS81/EME1, or SLX1/SLX4) to generate crossover or noncrossover products (180–184). Interestingly, in the absence of WRN, MUS81/EME1 promotes HR-dependent DSB repair by a poorly understood process that leads to chromosomal aberrations and appears to be error prone (167). SDSA is another alternative pathway for noncrossover resolution of recombination intermediates and a pathway for generating and extending D-loops. All RecQ helicases possess DNA SA activity (reviewed in Reference 8). More specifically, WRN enhances RAD52-mediated SA (53), and BLM stimulates DNA polymerase η -dependent SDSA (162) and is specifically required for SDSA in *Drosophila* (185). Because all RecQs possess SA activity, they could functionally complement one another at this step.

Thus, RecQ proteins interact with key enzymes or are themselves critical for proper DSB repair (Figures 2 and 3*b*). This observation could become clinically significant if synthetic lethal relationships involving RecQ helicases and DSB repair pathway components are identified or if therapeutic agents that complement or correct an inherited defect in one of the RecQ helicases are determined.

RecQ HELICASES IN DNA REPLICATION

Genome Replication

RecQ helicases participate in all phases of DNA replication (including initiation, Okazaki fragment processing, leading- and lagging-strand elongation, and resolution). Other replication-associated events in which RecQ helicases may play roles include interactions

with RPA, translesion DNA synthesis, replication fork restart, telomere replication, and intra-S-phase DNA damage signaling. Although the experimental and molecular details are not yet understood, specific roles for each RecQ helicase in DNA replication have been postulated (Figure 4).

RECQL4 and RECQL1 may play unique roles during initiation of DNA replication, but RECQL1's role is not well characterized (38), whereas RECQL4's role in initiation is better characterized in several species. The N-terminal region of xRTS, the *Xenopus* homolog of RECQL4, shares homology with Sld2, a yeast replication initiation protein (186, 187), and is essential for loading DNA Pol α onto replication origins (186). Similarly, RECQL4 plays a role during initiation of DNA replication in *Drosophila* and chicken DT40 cells (188–190), where it associates with replication origins during G₁/S phase (38) and interacts with replisome factors MCM10, MCM2-7 helicase, CDC45, and GINS (190, 191). MCM10 directly regulates the RECQL4 DNA helicase, and this interaction may be modulated by cyclin-dependent kinase phosphorylation (190). Consistent with these findings, homozygous deletion of *Recql4* is lethal during embryogenesis in mice and flies (76, 192).

FEN1 interacts with BLM, WRN, RECQL4, and RECQL5 (Figure 2), suggesting that RecQ helicases may play a role during maturation of Okazaki fragments (102, 193). However, whether RecQ helicases play complementary or redundant roles during lagging-strand DNA replication, or how they are coordinated, remains unknown.

Early research on cells from WS and BS patients suggested that replication fork movement might be slower in defective BLM and WRN cells than in controls because S phase lasts longer in these cells (194, 195). Later studies using fiber tracking analysis confirmed that defects in BLM or WRN decrease the rate of replication fork progression (196, 197). One explanation for this observation is that WRN and BLM resolve or remove DNA structures that impede fork movement (60, 104, 198), such as hairpins and G4 DNA (199, 200). Slower fork movement has also been observed in RECQL1-deficient (but not RECQL4-deficient) cells (38).

Chromosomal fragile sites (CFSs) are chromosomal regions that are prone to breakage, probably because they are inherently difficult to replicate (201, 202). WRN (203, 204) and BLM (205) strongly influence the stability of CFSs in normal cells (206). WRN specifically increases the ability of Pol δ to replicate through common fragile sites, such as FRA16D (207). RECQL1 also localizes to FRA16D and FRA3B in response to replicative stress (208) and may play a similar role in CFS DNA replication.

WRN can also stimulate bypass of DNA damage by translesion polymerases (209). WRN interacts with and stimulates translesion polymerases Pol η , Pol κ , Pol ι , and REV1 (210–212). Additionally, the WRN exonuclease can act as a proofreader for Pol η (211) and the replicative polymerase Pol δ (213). BLM stimulates primer extension by Pol η , but whether it promotes lesion bypass by Pol η is unknown.

RecQ helicases are thought to play important roles during replication restart after fork collapse. For example, defects in WRN, BLM, and RECQL1 increase the fragility of hot spots after replication fork stalling (203, 205), and single-molecule DNA fiber studies reveal

that DNA synthesis tracks are shorter in hydroxyurea-treated WRN-defective cells than in BLM-depleted or control cells (196). At the biochemical level, WRN strongly prefers substrates that mimic stalled replication forks (214–216), suggesting that WRN and BLM can convert a regressed fork via branch migration and/or degradation to a normal fork structure (Figure 4) (52, 217, 218).

Depletion of RECQL1 alters cell-cycle progression and induces replication-associated DSBs during S phase (219). Stable depletion of RECQL1 also renders cells sensitive to DNA damaging agents that directly or indirectly block replication fork progression (35, 38, 40, 219); activates phospho-CHK1; and induces accumulation of phospho-RPA, indicating replicative stress (219). RECQL1, like BLM, RECQL5, and WRN, promotes strand exchange on synthetic stalled replication fork-like structures (219, 220). RECQL1 and WRN, but not RECQL4 or RECQL5, unwind the leading strand of the replication fork. RPA modulates RECQL1 and WRN to favor unwinding over strand exchange and reannealing (219). Thus, RECQL1 and WRN behave similarly in vivo and in vitro in relation to replication fork-like structures with or without a leading-strand gap.

A unique role has been proposed for RECQL1 during replication restart after DNA damage (39), and this role is regulated by PARP1 (39, 221). In vitro RECQL1 helicase activity remodels chicken foot-like DNA substrates into replication fork-like molecules but fails to regress forks, and PARP1 inhibits this reaction (39). Single-molecule DNA fiber analysis revealed that RECQL1-deficient cells exposed to low-dose CPT fail to restart replication (39). Therefore, the current model proposes that PARP1 protects CPT-induced stalled forks from RECQL1 to limit premature fork restart and that RECQL1 plays a unique and essential role in fork restart (39). These results have significant clinical implications because CPT and other Topo inhibitors are widely used chemotherapeutic agents whose efficacy might be enhanced by combination with a small-molecule inhibitor of RECQL1.

In summary, depending on the type of DNA damage, BLM and WRN may stabilize replication forks, whereas RECQL1 and RECQL4 may play unique roles in replication fork restart or initiation. The precise roles of RECQL4 and RECQL5 in replication fork collapse or restart after DNA damage are unresolved and represent important areas for future research. RECQL5 might acquire a more important role in DNA replication as a backup in WRN-deficient cells (37). However, RECQL5 is also thought to inhibit transcription, thereby preventing fork collapse due to head-on collision between the transcription and replication machineries in a more proactive or preventive role (90; reviewed in References 88 and 222).

Resolution of Replication Intermediates and DNA Decatenation

During and after DNA replication, the parental and nascent DNA strands are linked together due to recombination between two molecules. The process of untangling the DNA strands, known as decatenation, usually occurs during the late S and G₂/M phases of the cell cycle. BLM directly suppresses aberrant recombination at stalled forks; therefore, in BLM-deficient cells there is a high degree of SCEs (223). This phenomenon is, in part, attributed to BLM's unique role in dissolution of late recombination intermediates, which is mediated by a protein complex, referred to as the BLM dissolvasome or BTR complex, composed of

BLM, Topo III α , and RMI1 and -2, (recently reviewed in References 3 and 224). This complex plays an important role in promoting dissolution, noncrossover outcomes from dHJs (see review in Reference 225). In yeast, the Sgs1/Topo III α /RMI1 protein complex has a similar function (3, 226).

RECQL5 physically interacts with Topo II α and specifically stimulates its DNA decatenation activity (227). Stable depletion of RECQL5 inhibits cell proliferation and induces a G₂/M checkpoint. BLM also interacts with Topo II α , which is important in preventing chromosomal breakage (228). These observations indicate that RecQ helicases play significant roles in the resolution of late replication intermediates and DNA decatenation.

Replication and Repair of Telomeres

Telomeres are protein–DNA structures at chromosome ends that preserve genome stability, survival, and proliferation at the cellular level and prevent degenerative diseases and cancer at the organismal level (229, 230). Human telomeres consist of ~10 kb of TTAGGG tandem repeats, a 3' ssDNA overhang, and a six-member shelterin protein complex (231, 232). Normal cell division causes the progressive loss of telomeric repeats, which are compensated for by telomerase; however, most human cells lack telomerase activity (233, 234). Critically short telomeres are recognized as chromosome breaks and trigger cell senescence or chromosome end-to-end fusions (235, 236). Telomere dysfunction contributes to the WS and BS pathologies in mouse models (69, 237). Telomeres are more sensitive to replicative stress than is the bulk genome (238, 239), which may partly explain the need for RecQ helicases in telomere replication.

WRN, BLM, and RECQL4 interact with shelterin proteins TRF1, TRF2, and POT1 (Figure 5) (73, 240–245). The shelterin proteins TRF1, TRF2, and POT1 generally stimulate unwinding of telomeric D-loops and forks by RecQ helicases (55, 73, 176, 240, 241, 244). WRN helicase and exonuclease activities cooperate to dissociate D-loops (55). TRF2 stimulates (242) and POT1 inhibits the WRN exonuclease on substrates containing a telomeric ssDNA overhang (246).

WRN-, BLM-, and RECQL4-deficient cells have distinct properties, but all are defective in telomere replication. WRN and RECQL4 localize to some telomeres during S phase (73, 247), and replication stress enhances telomeric localization of WRN (248). In contrast, BLM localizes to a subset of telomeres in either late S or G₂/M phase (43, 241). Thus, RecQ helicases may be differentially enriched at the telomere in a cell cycle–dependent manner, reflecting their different roles in telomere maintenance.

BLM and WRN play nonredundant roles in suppressing sister telomere loss (STL) and critical telomere shortening (43). In WRN-deficient cells, STL is more frequent in telomeres replicated from the G-rich lagging strand (247, 249), and WRN prevents large deletions during telomere replication (132). These data suggest that lagging-strand TTAGGG repeats are resistant to replication in the absence of WRN, possibly because G4s form (Figure 5). However, other evidence suggests that G4s cause telomere fragility rather than telomere loss, and telomere fragility was not reported in WRN-deficient cells but was observed in

RECQL4- and BLM-deficient cells (73, 238). Fragile telomeres manifest as multiple distinct telomeric spots at a single chromatid end (often a doublet) and result from treatments that cause breaks at CFSs (238). However, WRN and BLM unwind G4s, and RECQL4 does not (154, 250). Therefore, although BLM may unwind G4s and reduce telomere fragility, RECQL4 or WRN may disrupt other structures that cause telomere fragility or STL, respectively. The division of labor may relate to substrate specificity.

Regressed replication forks and D-loops can also interfere with telomere replication. Electron microscopy studies revealed that stalled replication forks at telomeric repeats spontaneously regress into four-way junctions (Figure 5) (251). Both WRN and BLM promote the formation and subsequent disruption of regressed replication forks (250, 252), whereas unwinding of four-way junctions by RECQL4 has not been observed (154). By contrast, all three helicases can disrupt telomeric D-loops (55, 73, 177, 179, 253). Intratelomeric D-loops form naturally when the 3' ssDNA telomeric overhang wraps around and invades the duplex telomeric sequence, forming a large T-loop that is stabilized by the D-loop (Figure 5) (254, 255). Failure to resolve this structure can cause T-loop cleavage and dramatic telomere shortening, which may explain why cells express several helicases that can dismantle the telomeric D-loop (243, 256, 257). Interestingly, not all telomeres are affected equally in BLM- and WRN-deficient cells, but loss of both proteins considerably increases the number of STLs (43).

RecQ helicases may also disrupt intertelomeric D-loops generated by aberrant strand invasions of the 3' telomeric overhang. This would prevent inappropriate recombination at telomeres and is consistent with cellular observations that WRN, BLM, or RECQL4 deficiency increases SCEs at telomeres (Figure 5) (73, 258). HR is suppressed at telomeres in normal cells but is elevated in cancer cells that use alternative lengthening of telomeres (ALT) to prevent telomere attrition during cell division (259). Evidence indicates that WRN and BLM dissociate intermediates of the ALT pathway (260–262). Thus, RecQ helicases exhibit a division of labor at telomeres by disrupting intermediate structures to (a) prevent telomeric HR in normal cells or (b) facilitate completion of the ALT pathway in cancer cells.

The roles of RECQL1 and RECQL5 in telomere maintenance are poorly characterized. However, RECQL5 does not unwind telomeric D-loops (73). *Recql1*-knockout mice have normal length telomeres (33). Notably, simultaneous knockout of *C. elegans rtel-1* and *recql5* causes synthetic lethality due to elevated recombination (263). *Rtel-1* is the *C. elegans* homolog of human RTEL1, a helicase that plays a significant role in regulating telomere length (256, 264), which implies that RECQL5 may have an uncharacterized role in telomere maintenance in human cells.

RecQ Helicases, BRCA1, and the Fanconi's Anemia Pathway

RecQ helicases may also engage in cross talk with BRCA1 and the Fanconi's anemia (FA) core complex. Both WRN and BRCA1 are thought to participate in repair of interstrand cross-links (170), the primary targets of the FA pathway. BLM also forms foci that colocalize with the proliferating cell nuclear antigen and the BRCA1-associated genome surveillance complex (265). FANCM, a member of this complex, is required for formation

of BLM nuclear foci after replication stalling, and it helps prevent chromosome instability (266). FANCD2 and FANCD1 interact with BLM and colocalize in DNA damage-induced nuclear foci, and BLM stability correlates positively with expression of FANCD2 and FANCD1 (267, 268). Further characterization of the RecQ proteins and the FA pathway is an important area for additional research.

S-Phase Checkpoint

The intra-S-phase checkpoint is activated to maintain DNA replication fork stability, promote fork recovery, and facilitate fork restart. Ataxia-telangiectasia- and RAD3-related protein kinase (ATR) and ataxia-telangiectasia-mutated protein kinase (ATM) play critical but distinct roles in this process. Depending on the type of damage, both are required to recover from replication-induced DSBs (269). RecQ helicases play a limited role in the intra-S-phase checkpoint (270). However, the checkpoint kinases ATM and ATR are not fully activated in WRN-deficient cells exposed to CPT (271, 272), which leads to replication fork collapse (271) and DSBs that are subsequently repaired by recombination (167).

Depletion of RAD9, which disrupts RAD911, eliminates recruitment of WRN to CPT- and hydroxyurea-induced DNA damage and prevents ATR/ATM-dependent serine and tyrosine phosphorylation of WRN (273). The current model is that WRN participates in the intra-S-phase checkpoint in response to replication-dependent DSBs (274, 275); the RAD911 complex recruits WRN, and ATR phosphorylation of WRN stabilizes WRN at stalled replication forks. Finally, ATM may dissociate WRN from DSBs to allow binding and initiation of RAD51-dependent recombination repair (270). Studies also show that BLM plays a role in recovery from S-phase arrest in response to hydroxyurea and that ATR plays a role in the regulation of BLM function (276). Posttranslational modifications, such as phosphorylation, are known to modulate the catalytic activities of the RecQ proteins (see the sidebar titled Posttranslational Modifications of WRN).

RECQL1-depleted cells activate the intra-S-phase checkpoint normally (219), and RECQL5-depleted cells accumulate in late S phase (227). Thus, these RecQ helicases are not required for intra-S-phase arrest. RECQL4 may play a role in the intra-S-phase checkpoint because RECQL4-deficient or -defective cells fail to arrest in S phase after UV, hydroxyurea, or ionizing radiation treatment (172, 277). However, the molecular details and/or mechanisms are not yet known.

TOPOISOMERASES AND RecQ HELICASES

BLM interacts with and reciprocally stimulates Topo I on RNA–DNA hybrids (278). BLM stimulates ribosomal transcription, and the BLM–Topo I interaction may modulate transcription of nucleolar ribosomal DNA (278). WRN also interacts with Topo I and stimulates Topo I-catalyzed relaxation of negatively supercoiled DNA (279). Furthermore, it protects against the deleterious effects of Topo I inhibitors (197, 279–281). WRN stimulates ribosomal transcription, but whether this action is mediated by an interaction with Topo I is unknown (282). Collectively, there are multiple Topo–RecQ connections, which may contribute to the faithful segregation of chromosomes.

POSTTRANSLATIONAL MODIFICATIONS OF WRN

Multiple posttranslational modifications (PTMs) regulate the catalytic activities, cellular localization, and protein–protein interactions of RecQ helicases. For example, WRN is phosphorylated by ATM, ATR, DNA-PKcs, and c-Abl tyrosine kinase (271, 272, 275, 300). Suppression of ATR-mediated phosphorylation in vivo alters the localization of WRN to nuclear foci and its colocalization with RPA, and can lead to breakage of stalled replication forks (275). Serine/threonine phosphorylation of WRN by DNA-PKcs and tyrosine phosphorylation by c-Abl kinase, in vitro, inhibit the WRN exonuclease and helicase (126, 300). Oxidation also inhibits both WRN activities (301). WRN is also acetylated in vivo and in vitro by p300, a lysine acetyltransferase, and acetylation stimulates the helicase and exonuclease, possibly enhancing the role of WRN in BER (302). Acetylation of WRN by p300 causes translocation from nucleolar to nuclear foci (303). WRN also associates with SUMO-1 (304), but the functional consequence of SUMO modification of WRN remains to be established. Clearly, understanding PTMs for each of the RecQs is an important line of research.

RecQ HELICASES AS THERAPEUTIC TARGETS

RecQ helicases are dysregulated in cancer (283); therefore, they represent novel therapeutic targets. Recently, small-molecule inhibitors of both WRN and BLM have been identified (284, 285). Furthermore, when cells defective in the FA pathway were treated with a WRN inhibitor, the response to mitomycin C was abrogated (286). As described above, RecQ helicases play a critical role in the response to DNA damage; thus, combination therapies that target one of the RecQ helicases might induce synthetic lethality. For example, certain oncogenes such as *Myc*, which are frequently mutated and overexpressed in cancers, are synthetically lethal when WRN is inactivated due to incipient replication catastrophe (287). Although RecQ-targeted drugs may be useful for therapy, they are also of interest as tools for cell-based studies.

INTERACTIONS BETWEEN RecQ HELICASES

Early studies in human cells reported, with some surprise, the discovery of five distinct RecQ helicase–related enzymes, initiating an ongoing debate about whether the activities and functions of the five human RecQ helicases are complementary or redundant. After extensive research, this question remains unresolved, although some insights have been gained (Figures 2 and 6). Most importantly, significant functional interactions between the RecQ helicases have been documented. Although WRN and BLM share similar substrate specificity (250), BLM inhibits the WRN exonuclease (288), and RECQL4 specifically stimulates BLM (289). BLM promotes retention of RECQL4 at DSBs in vivo (289), and cells defective in BLM and RECQL4 proliferate slowly with elevated SCEs. Cells that are defective in WRN and RECQL5 demonstrate synthetic lethality (37). In vivo RECQL5 dissociates slowly from DSBs in WS and BS fibro-blasts. However, RECQL5 specifically stimulates WRN but not BLM, and RECQL5 and WRN cooperate during repair and restart of synthetic stalled replication fork–like structures (37). These results suggest that RECQL5 and WRN play cooperative and complementary roles. Additional research on the

interactions between RecQ helicases is needed, and these interactions remain of great interest in the field.

CONCLUDING REMARKS

RecQ helicases play important roles in DNA repair, recombination, and replication that are mediated primarily through interactions with proteins that regulate or play critical roles in these pathways (Figure 2). Recently, it has become clear that RecQ helicases also play direct roles in these DNA metabolic pathways. The biological importance of RecQ helicases is well established; however, whether they can be exploited as therapeutic targets, or whether methods can be developed to treat or prevent illness in patients who carry modified alleles of BLM, WRN, or RECQL4, is not yet clear.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Miriam Sander (Page One Editorial Services, Boulder, Colorado) for her contribution to this review as scientific writer/editor. We thank Drs. Robert Brosh, Tomasz Kulikowicz, and Raghavendra Shamanna for their comments. We also thank Thomas Wynn for his excellent artwork. We apologize to our colleagues whose work is not cited in the review due to space limitations. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute on Aging. Support was also provided by the Scaife Foundations through the Center for Nucleic Acid Technology to P.L.O.

Glossary

Translocate	refers to the directional movement of helicases in a 5' → 3' or 3' → 5' direction along DNA
RQC domain	RecQ C-terminal domain
HRDC domain	helicase and RNase D-like C-terminal domain
G quadruplex (G4)	a four-stranded nucleic acid structure stabilized by stacked non-Watson-Crick Hoogsteen base pairs between four planar-orientated guanosine nucleotides
SCE	sister chromatid exchange
HR	homologous recombination
CPT	camptothecin
BER	base excision repair
NER	nucleotide excision repair
NHEJ	nonhomologous end joining
Alt-NHEJ	alternative nonhomologous end joining
Nucleoprotein filament	a critical intermediate in HR in which eukaryotic recombinase RAD51 coats ssDNA and initiates a homology search

Synthetic lethality	cell death resulting from inactivation or inhibition of two gene products that are nonlethal when inactivated individually
Shelterin	a six-protein complex that localizes to the terminal TTAGGG repeats of mammalian telomeres
T-loop	a structure in which telomeric ssDNA at the chromosome terminus invades and hybridizes to the complementary strand of adjacent telomeric dsDNA
Intra-S-phase checkpoint	a mechanism that aborts or slows replication and cell-cycle progression through S phase in cells with DNA damage

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SUMMARY POINTS

1. Each of the five human RecQ helicases can unwind forked dsDNA and anneal ssDNA, and RecQ helicases play critical roles in all aspects of DNA metabolism, including DNA repair, recombination, replication, and transcription.
2. There is a division of labor among the human RecQ helicases, and each has at least one specialized biological function, but collectively they all contribute widely to genome stability.
3. Human RecQ helicases interact with each other, and their roles in different DNA metabolic pathways can be both overlapping and complementary, creating a functional complexity that is a challenge to decipher.
4. Mutations in BLM, WRN, and RECQL4 are associated with heritable disease syndromes and susceptibility to cancer.
5. Because RecQ helicases play diverse roles in the response to DNA damage, they have potential as novel cancer chemotherapeutic targets.

FUTURE ISSUES

1. Additional analyses of the structure–function relationships among human RecQ helicases are warranted because they could yield insight into their shared and unique properties. For example, there is no recognizable winged helix in RECQL5, so does it interact with DNA differently than do the other family members?
2. Additional novel and disease-linked mutant alleles of BLM, WRN, and RECQL4 should be characterized to better understand the molecular basis of the clinical manifestations of BS, WS, BGS, RTS, and RAPADILINO syndrome. Curiously, WRN deficiency is associated with an aging-specific phenotype, and it is important to understand how the separate activities of its helicase and exonuclease cooperate in biological pathways.
3. The role of the RecQ helicases in the context of the cellular DNA damage response network should continue to be investigated because of the critical role played by the DNA damage response in preserving genome stability.
4. The RecQ proteins, and their interaction partners, are posttranslationally modified by phosphorylation, acetylation, and SUMO-ylation. These and other PTMs are critically important regulators of the enzymes' functions, and additional research on this subject is needed.
5. Small-molecule inhibitors and enhancers for each RecQ helicase should be developed because (a) such compounds could potentially enhance the efficacy of chemotherapeutic agents already in clinical use; (b) they might also prove beneficial in other clinical contexts; and (c) specific protein inhibitors and activators will be useful in studying RecQ helicase mechanisms and functions.

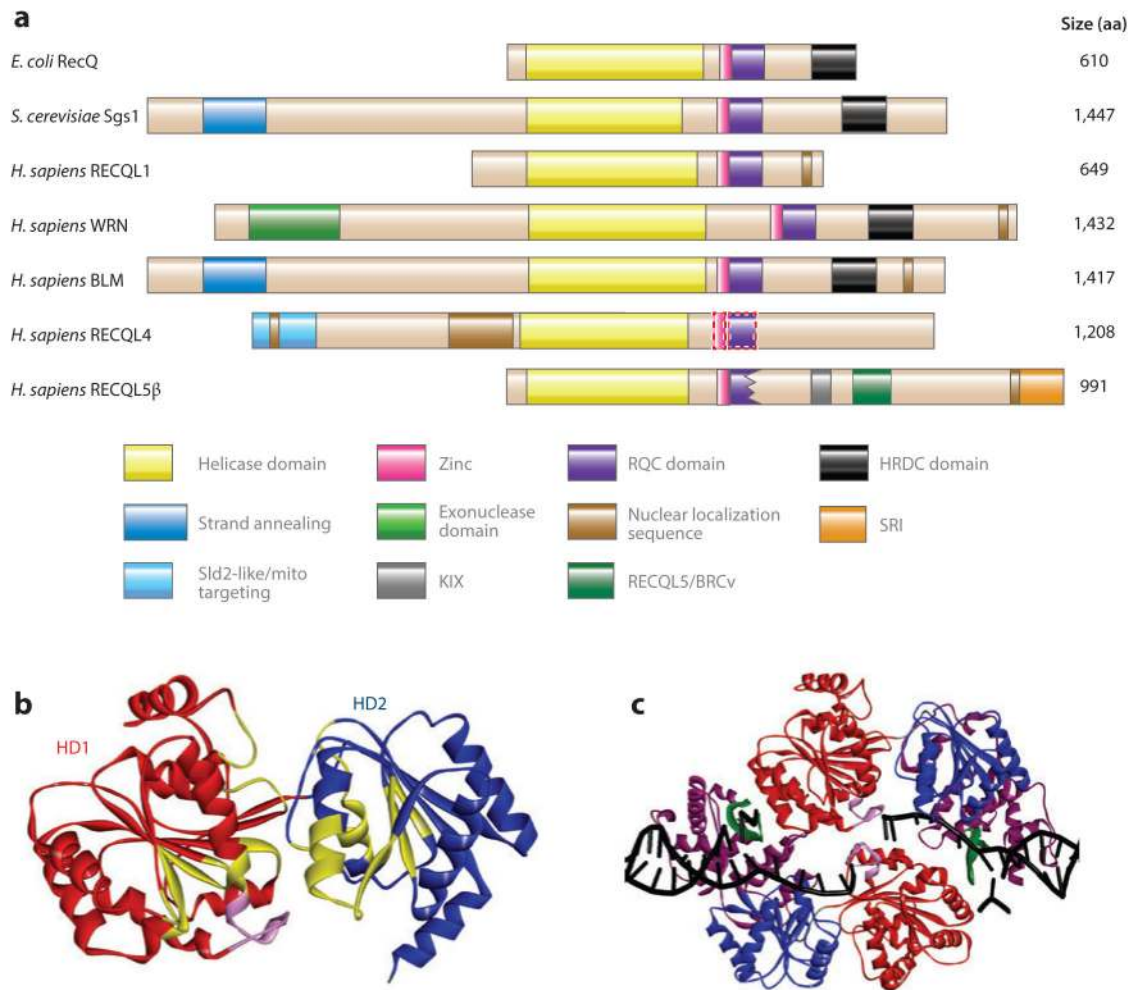


Figure 1.

RecQ helicase protein family. (a) The domain structure of RecQ helicases from *Escherichia coli*, *Saccharomyces cerevisiae*, and *Homo sapiens*. Homologous domains are represented with solid-color boxes outlined with solid lines; the tentatively assigned zinc and RQC domains of RECQL4 are represented by rectangles outlined with dotted lines. (b) The helicase domains of human RECQL1, based on Protein Data Bank (PDB) identifier 2WWY (24). The structure includes two RecA-like domains, helicase domains 1 [HD1 (*red*)] and 2 [HD2 (*blue*)], and an ATP-binding cleft. The conserved helicase motifs are in yellow, and the aromatic loop is in pink. (c) The truncated RECQL1 bound to DNA is based on PDB 2WWY. The RQC domain is in purple, the β -hairpin is in green, and the DNA is in black. Abbreviation: aa, amino acid.

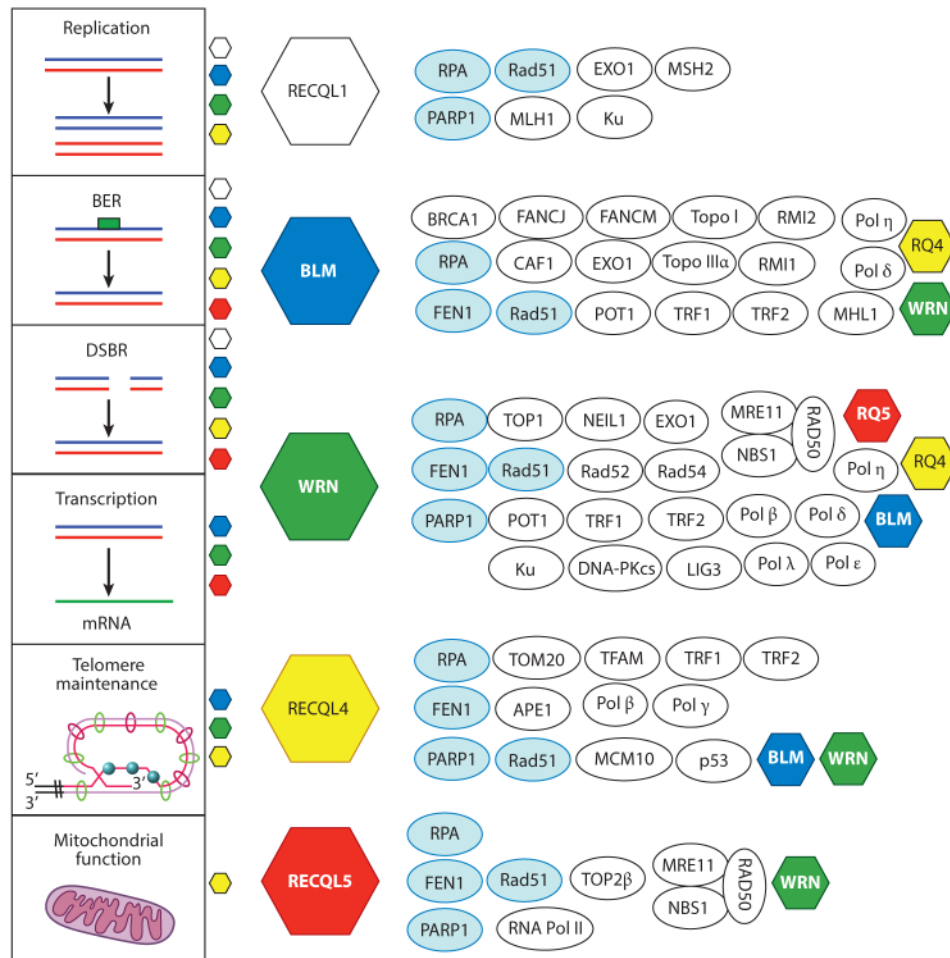


Figure 2. Protein-protein interactions among the RecQ helicases. The vertical panel to the left lists six DNA metabolic pathways and the RecQ helicases that participate in those pathways. Each RecQ helicase is represented as a colored hexagon. The interacting proteins are shown next to each RecQ helicase. Proteins that interact with four or five RecQ helicases are represented by light blue ovals, and proteins that interact with two or more RecQ helicases are represented by white ovals. Abbreviations: BER, base excision repair; DSBR, double-strand break repair; mRNA, messenger RNA; Topo, topoisomerase.

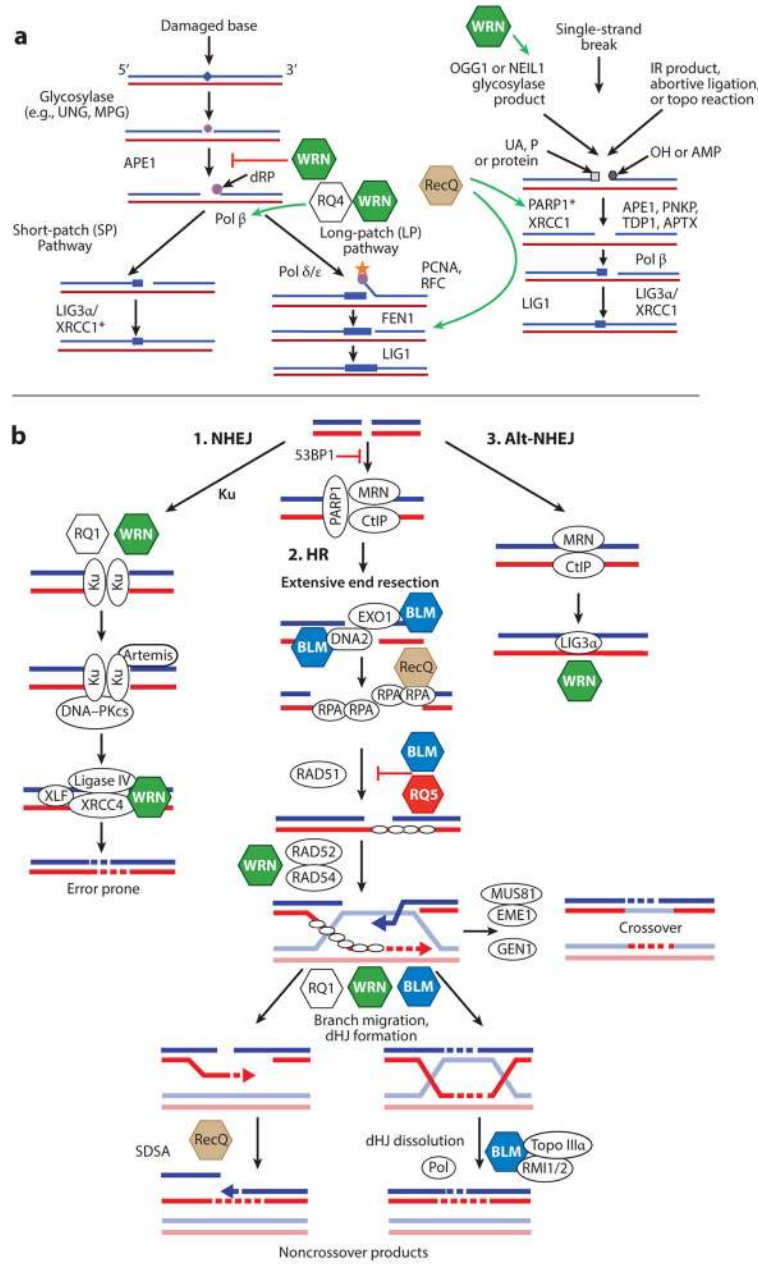


Figure 3. RecQ helicases in base excision repair (BER) and double-strand break repair (DSBR). (a) Summary of the BER pathway. Interactions between RecQ helicases and BER proteins are depicted by a green arrow (for activation) or a red bar (for inhibition). Asterisks after *PARP1* and *XRCC1* indicate that these genes are specifically downregulated by RECQL5 loss. The brown RecQ hexagon indicates that multiple RecQs interact with these proteins. (b) Summary of DSBR pathways. (i) Nonhomologous end joining (NHEJ). (ii) Homologous recombination (HR). (iii) Alternative (Alt)-NHEJ. The involvement of RecQ helicases in DSBR is depicted. Each RecQ helicase is represented as a colored hexagon. The colored hexagons are consistent with the coloring in Figure 2. Other key proteins are shown

as white ovals. DNA molecules are shown as blue/red or light blue/red duplexes. Dashed lines denote nascent DNA synthesis. Abbreviations: dHJ, double Holliday junction; dRP, deoxyribose phosphate; IR, ionizing radiation; MPG, methylpurine DNA glycosylase; P, 3'-phosphate; PCNA, proliferating cell nuclear antigen; Pol, DNA polymerase; RFC, replication factor C; SDSA, synthesis-dependent strand annealing; Topo, topoisomerase; UA, 3'- α,β unsaturated aldehyde; UNG, uracil DNA glycosylase.

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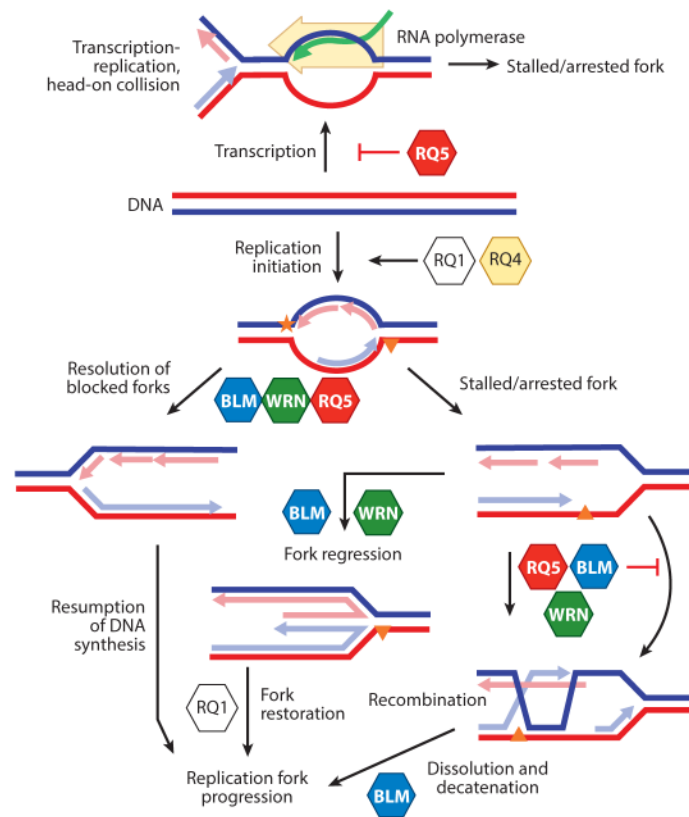


Figure 4. RecQ helicases in DNA replication. Putative roles of RecQ helicases during initiation of DNA replication, Okazaki fragment processing, leading- and lagging-strand elongation, fork restart, and regression are indicated. The orange star and triangle denote polymerase blocking DNA damage. In addition, RECQL5 may specifically inhibit transcription to prevent replication and transcription fork collisions. Abbreviations: RQ1, RECQL1 helicase; RQ4, RECQL4 helicase; RQ5, RECQL5 helicase.

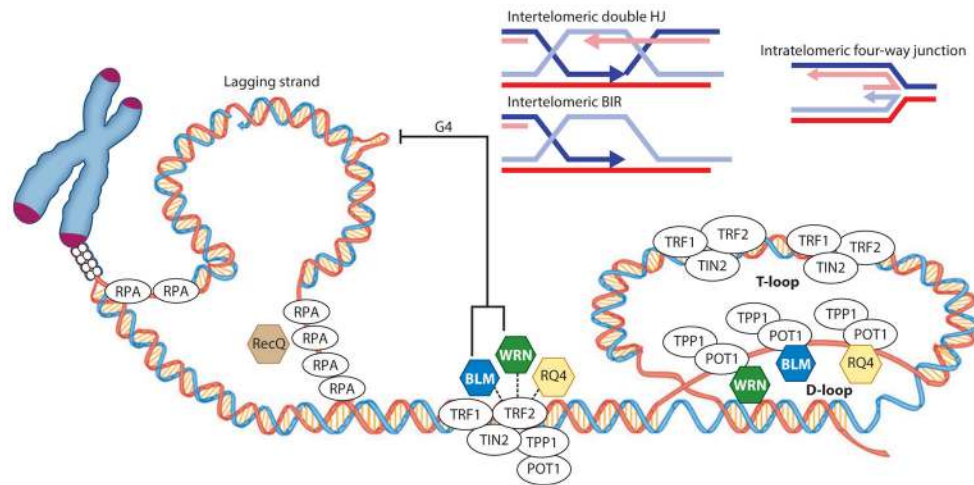


Figure 5.

RecQ helicases (BLM, WRN, and RECQL4) in telomere maintenance and repair. Interactions with shelterin proteins (TRF1, TRF2, and POT1) and resolution of secondary structures that are potential barriers to telomere replication are shown. RecQs may resolve inter- and intratelomeric substrates, G quadruplexes (G4s), and telomeric T-loops and D-loops. Abbreviations: BIR, break-induced replication; HJ, Holliday junction; RPA, replication protein A; RQ4, RECQL4; TIN2, TRF1-interacting nuclear factor 2; TPP1, POT1-interacting telomere end-binding protein.

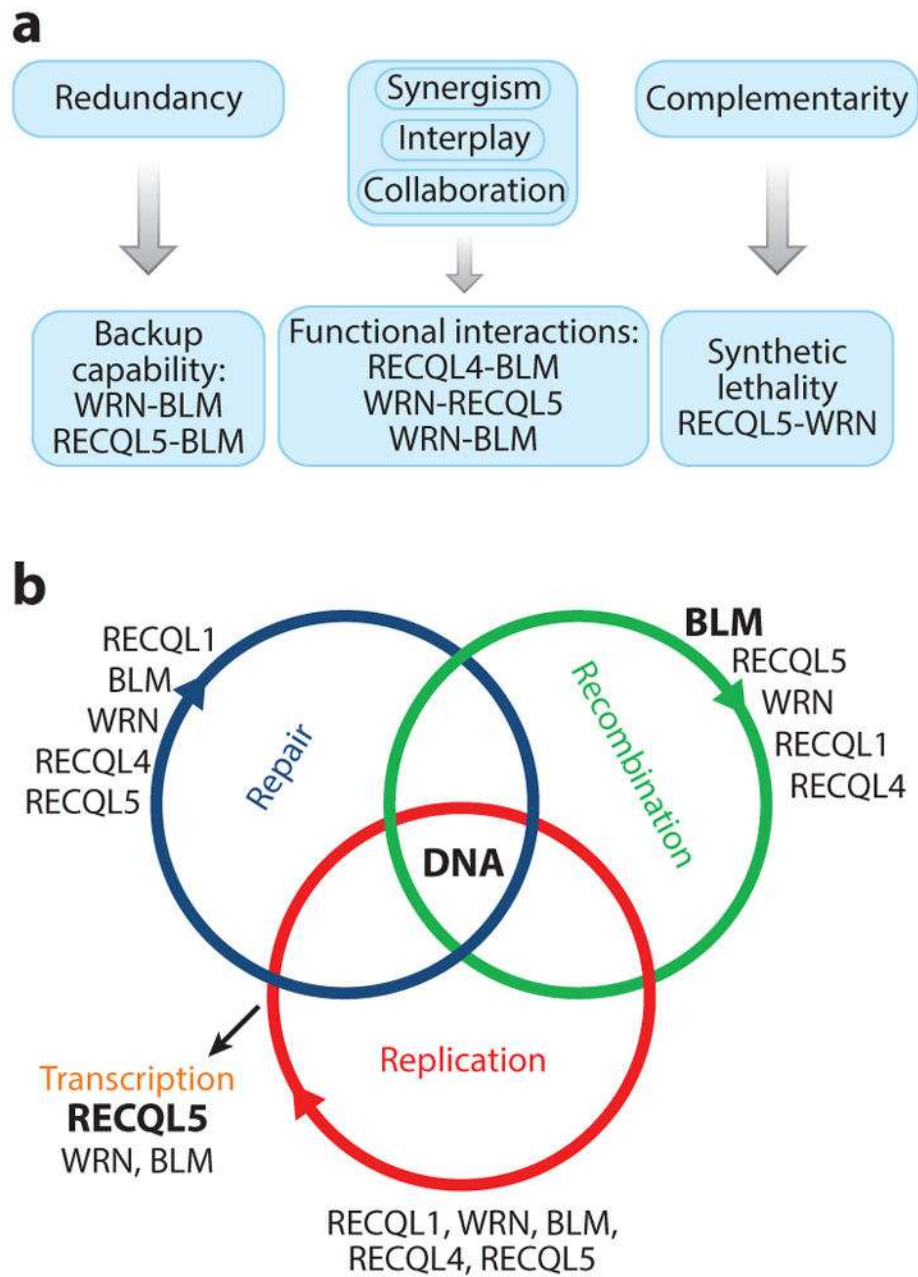











Figure 6. Interactions between RecQ helicases. (a) Human RecQ helicases can play both overlapping and nonredundant roles. They can cooperate with each other functionally and complement each other in certain DNA metabolic pathways. (b) The predominant role of each RecQ helicase in each DNA metabolic pathway is indicated. Larger, bold font equates to greater predominance for the role of a specific enzyme in a specific pathway.

Table 1 Comparison between the relative helicase activity of *Escherichia coli* RecQ and that of the five human RecQ helicases^a

Substrates ^b	RecQ ^c	RECQL1	BLM	WRN	RECQL4 ^d	RECQL5
	+	-	-	-	+/-	ND
	+	++	+	+	+	+
	+	++	~/~	~/~	ND	ND
	ND	+	+	+	-	-
	ND	~/~	+	+++	-	-
	+	-	+++	+++	-	-

Substrates ^b	RecQ ^c	RECQL1	BLM	WRN	RECQL4 ^d	RECQL5
	+	+	+++	+++	-	~
	ND	-	+	+	ND	ND
						

^aData are from the following published studies: References 11, 151, 154, 253, and 290–294.

^bDNA, red and blue; RNA, green.

^cRelative helicase activity: +, weak; ++, moderate; +++, strong; -, no activity; ~/-, partial activity; ND, not determined.

^dRECQL4 activity in the absence of ssDNA.