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Distinct Roles of RECQ1 in the Maintenance of Genomic Stability

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Abstract

Five human RecQ helicases (WRN, BLM, RECQ4, RECQ5, RECQ1) exist in humans. Of these, three are genetically linked to diseases of premature aging and/or cancer. Neither RECQ1 nor RECQ5 has yet been implicated in a human disease. However, cellular studies and genetic analyses of model organisms indicate that RECQ1 (and RECQ5) play an important role in the maintenance of genomic stability. Biochemical studies of purified RECQ1 protein demonstrate that the enzyme catalyzes DNA unwinding and strand annealing, and these activities are likely to be important for its role in DNA repair. RECQ1 also physically and functionally interacts with proteins involved in genetic recombination. In this review, we will summarize our current knowledge of RECQ1 roles in cellular nucleic acid metabolism and propose avenues of investigation for future studies.

Keywords

RECQ1 (RECQL); RecQ; helicase; genomic stability; DNA repair

Introduction

RECQL or RECQL1 (hereafter designated RECQ1) belongs to a class of DExH-containing DNA helicases that have been implicated in diseases of premature aging, cancer, and chromosomal instability (for review, see [1,2]). These include Werner syndrome, Bloom's syndrome, and Rothmund-Thomson syndrome that have mutations in the *WRN*, *BLM*, and *RECQ4* genes, respectively. Although the clinical importance of RECQ1 is yet to be fully appreciated, it is becoming increasingly apparent that RECQ1, the first of the human RecQ helicases to be discovered, has unique and important roles in cellular DNA metabolism. RECQ1 is the most abundant of the five human RecQ helicases, and RECQ1 depletion studies indicate its importance in human cells for chromosomal stability. Genetic studies of model organisms with mutations in genes encoding proteins with sequence homology to RECQ1 also demonstrate its importance in genome homeostasis. Advances in understanding the structural and biochemical properties of RECQ1 have led to further insights to its proposed molecular functions. These topics will be discussed to provide a conceptual basis for how RECQ1 might help cells to cope with DNA damage. We also suggest important avenues for RECQ1 research that may help in understanding one of the less characterized human RecQ helicases.

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RECQ1-like Helicases and Their Genetic Functions in Model Organisms

Close inspection of the RECQ1 amino acid sequence reveals similarity in the helicase core domain to a number of RECQ1-like proteins from different species, as shown in Fig. 1A. In addition to the helicase core domain containing the seven conserved amino acid motifs, two additional regions can be found in a number of the RecQ helicases [3], including the RECQ1-like helicases. The RecQ C-terminal region (RQC) that has been implicated in protein interactions and DNA binding is present in all the RECQ1-like proteins shown. However, the Helicase and RNase D C-terminal Domain (HRDC), necessary for the function of BLM in Holliday Junction (HJ) dissolution and important for DNA structure binding [4], is absent in human and mouse RECQ1, but can be found in RECQ1-like helicases for certain species. The human *RECQ1* gene was identified by two groups [5,6] a decade after the discovery of the *E. coli RecQ* gene [7]. Phylogenetic analysis of the RECQ1-like helicases reveals that human RECQ1 is most closely related by sequence to mouse and chicken (Fig. 1B).

Cellular studies demonstrate that RECQ1 plays an important role in human and mouse for chromosomal stability and the DNA damage response ([8], and discussed below). The use of model genetic systems have enable researchers to study the functions of RECQ1-like helicases in whole organisms, and in some cases suggests diverse physiological roles. Because chicken B-lymphocyte DT40 cells are genetically easy to manipulate, they have been widely used to study DNA repair genes, including those encoding the RecQ helicases [9]. Although *recq1* single knockout DT40 cells were not significantly different from the wild-type cells in cell growth, sensitivity to methyl methanesulfonate (MMS), or sister chromatid exchange (SCE) frequency, *recq1 blm* double knockout DT40 cells grew slower than *blm* single knockout cells due to an increased population of dead cells [10]. A higher incidence of SCE was observed in mitomycin C-treated *recq1 blm* mutant cells compared to *blm* cells (Wang et al., 2003), suggesting that *recq1* can compensate for a *blm* deficiency. However, Otsuki et al. reported that disruption of the *recq1* gene in a *req5 blm* mutant background did not affect UV or MMS survival, UV-induced SCE, or the frequency of damage-induced mitotic chiasma, leading the authors to conclude that RECQ1 might not function in DNA replication or repair in chicken cells [11].

In *Neurospora crassa*, two helicases (QDE3, RecQ-2) sharing sequence similarity to RECQ1 exist, and both are important for the DNA damage response [12,13]. Mutation in the gene encoding the RECQ1-like helicase QDE3, an RNA-dependent RNA polymerase (QDE1), or an Argonaute protein containing a PIWI domain (QDE2) result in a quelling defect in RNA silencing [14-16]. QDE3 alone is involved in a key step of activation and maintenance of RNA silencing [17]. Recently, a novel small RNA (qiRNA) was identified on the basis of its interaction with QDE2 [18]. The newly found qiRNA is 20-21 nucleotides long with a strong preference for uridine at the 5' end, and mostly from the ribosomal DNA locus. It is likely that this qiRNA is the counterpart of mammalian piRNA (for review, see [19,20]), which was first observed in fruit fly to be associated with PIWI protein, QDE2 in *Neurospora crassa*. Interestingly, the *qde3* mutation abolished qiRNA production, indicating that QDE3 is required for qiRNA biogenesis [18]. Consistent with the *Neurospora* genetic results, RECQ1 was found in a piRNA complex isolated from rat testis [21]. The piRNA protein complex contained ATPase and DNA helicase activities as well as RNA cleavage activity that would be predicted to be catalyzed respectively by RECQ1 and a conserved Argonaute protein responsible for RNA-guided cleavage of target RNAs.

Proteins sharing sequence similarity with RECQ1 have been identified in the plants *O. sativa* and *A. thaliani* (Fig. 1A). Expression of rice RecQ1 increased with exposure to various DNA damaging agents, suggesting that RecQ1 may be involved in DNA repair [22]. In addition to its role in the DNA damage response, rice RECQ1 is required for RNA silencing induced by particle bombardment for inverted-repeat DNA, which is likely formed by transposon

elements [23]. It has been proposed that mammalian RecQ helicases might also have a function in gene silencing, but studies using mouse models for Wrm, Blm, and RecQ1 suggest that they are not essential for sequence-specific mRNA degradation in response to dsRNA [24]. It is possible that certain mammalian RecQ helicases are involved in the production of small RNA molecules. With novel small RNAs and gene silencing pathways being discovered, the importance of mammalian RECQ1-like helicases in defending genome integrity through gene silencing remains to be determined.

Prospective Importance of RECQ1 in Mammalian Cells

Although a human disease has not yet been genetically linked to mutations in the *RECQ1* gene, cellular phenotypes associated with RECQ1 deficiency in mouse and human indicate that RECQ1 has a uniquely important role in genomic integrity. We will summarize what is known about the cellular importance of RECQ1, pointing out some hypotheses for the functions of RECQ1 that will be considered more extensively at the molecular level in the section entitled *Structural Features and Biochemical Functions of RECQ1*.

Chromosomal Instability in RECQ1-Deficient Cells

Primary embryonic fibroblasts from *Recql*-null mice display aneuploidy, spontaneous chromosomal breakage, frequent translocation events, and elevated SCE [25]. In RECQ1-deficient human [26] and mouse [25] cells there is an increased load of DNA damage as exemplified by the accumulation of γ H2AX foci, a marker of double strand breaks. Transient knockdown of RECQ1 in human cells resulted in significantly elevated spontaneous SCE [26]. It is plausible that a role of RECQ1 in homologous recombinational repair helps cells to cope with strand breaks that arise directly from DNA damage or are a consequence of broken replication forks at sites of replication blockage [8]. An alternative hypothesis is that RECQ1 helps cells to circumvent the consequences of DNA damage that may occur at replication forks. However, given the genetic evidence that RECQ1-deficient cells are sensitive to ionizing radiation that introduces strand breaks [25,26], we favor the hypothesis that RECQ1 plays a direct role in the repair of strand breaks and hence the maintenance of chromosomal stability through a mechanism that is yet to be fully understood.

A platform for understanding the role of RECQ1 in SCE suppression begins with understanding how the sequence-related BLM helicase suppresses SCE. In human cells, BLM physically interacts with Top3 α , and the two proteins together have the ability to catalyze double HJ dissolution on model DNA substrates in a reaction that requires BLM-mediated ATP hydrolysis and the active-site tyrosine residue of Top3 α [27]. This reaction gave rise exclusively to non-cross-over products, as predicted from the hemicatenane model, and supports a proposed role of BLM with Top3 α as a suppressor of SCEs. RMI1 (BLAP75) promotes this BLM-dependent dissolution of the homologous recombination (HR) intermediate by recruiting Top3 α to the double HJ [28,29]. Interestingly, BLM appears to be unique in the double HJ dissolution reaction since WRN, RECQ1 and RECQ5 all failed to substitute for BLM [4,28]. Moreover, association of Top3 α and BLAP75 with BLM stimulates its HJ unwinding activity; however, neither WRN nor *E. coli* RecQ HJ unwinding was stimulated by Top3 α /BLAP75 [30]. Very recently, a new component of the BLM-Top3 α complex, designated RMI2, was identified that is important for the stability of the BLM protein complex [31,32]. RMI2 deficiency in vertebrate cells results in chromosomal instability [31,32], suggesting its function as a tumor suppressor. RMI2 enhanced the double HJ dissolvase activity of the BLM-Top3 α complex [31], indicating that additional proteins are likely to be involved. In fact, other proteins were isolated with the RMI2 complex, including the mismatch repair complex MSH2/6, RPA, and the Fanconi Anemia proteins FANCM and FAAP24 [31].

The suppression of recombinant cross-over products that are detected as sister chromatid exchanges is thought to be specific to the coordinate functions of yeast Sgs1 and Top3, and its human counterparts, BLM and Top3 α . However, RECQ5 and RECQ1 also interact with Top3 α [33,34], and elevated SCE is also found in fibroblasts from RECQ5 [35] or RECQ1 [25] knockout mice as well as human cells depleted of RECQ1 by RNA interference [26]. These studies suggest that RecQ helicases participate in non-redundant pathways to suppress cross-overs during mitosis [8].

Although RECQ1 was not observed *in vitro* to substitute for BLM in the BLM-Top3 α complex double HJ dissolution reaction [4], RECQ1 may interact with Top3 α in a related protein complex with additional factors and perform a function important for genomic stability. Conceivably, in a BLM-impaired condition, RECQ1 or another RecQ helicase may partially substitute for BLM through its protein partnership with a topoisomerase.

A thorough investigation of RECQ1 protein interactions and their biological significance would likely provide insight to the potentially multiple roles of RECQ1 in DNA repair, one of which may be double strand break repair by a putative function in DNA end-processing (see *RECQ1 Protein Interactions*). Although RECQ1 may play a role in modulating homologous recombinational repair, a function of RECQ1 in nonhomologous end-joining, a major pathway for repair of double strand breaks throughout the cell cycle in mammalian cells [36], is also a possibility.

RECQ1 is Required for Normal Proliferation in Human Cells

HeLa cells transfected with control- or RECQ1-shRNA plasmid were found to be impaired for cell growth by a colony forming assay [26]. RECQ1 deficiency in transformed HeLa cells led to perturbation of normal cell cycle progression and compromised cellular ability to maintain G₂/M checkpoint [26]. In an independent study, silencing RECQ1 expression through RNA interference was observed to induce mitotic catastrophe specifically in growing cancer cells since normal human fibroblasts were refractory to the anti-proliferative effects of siRNA-RECQ1 [37]. Local and systemic administration of RECQ1-siRNA mixed with polyethyleneimine polymer or cationic liposomes prevented cancer cell proliferation in mouse models of cancer without detectable side effects [38]. Based on their findings, the authors suggested that RECQ1-siRNA formulated with a cationic liposome and introduced through intravenous injection may be a useful approach to target specific cancers.

The high copy number of RECQ1 in cancer cells may provide a growth advantage in certain human tumors [37,39]. Mitotic death of cancer cells induced by RECQ1 silencing or RECQ1-specific small molecule inhibitors is a potential strategy for anti-cancer therapy, as suggested for other helicases [40,41].

Structural Features and Biochemical Functions of RECQ1

Recently, the crystal structure of a truncated form of RECQ1 was solved, representing an important advance in the field. Biochemical studies characterized the DNA branch migration activity of RECQ1. Analyses of RECQ1 helicase and strand annealing activities revealed a better understanding of its interactions with DNA substrates and some differences in substrate specificity of RECQ1 compared to the other RecQ helicases. Finally, studies of RECQ1 protein interactions and advances in understanding the functions of RECQ1 protein partners have led to new hypotheses for the cellular role of RECQ1.

Structure of Truncated RECQ1

A crystal structure of a catalytically active truncated RECQ1 (RECQ1^{T1}, residues 49–616 (of 649) of RECQ1, followed by a C-terminal tag of 22 amino acids) with ADP-Mg²⁺ complex

was reported [42]. The overall structure of RECQ1^{T1} was similar to that of *E. coli* RecQ [43] for both the core helicase domain and the Zn²⁺ binding domain residing just after the helicase core. However, the Winged Helix (WH) domain in the C-terminal region of RECQ1^{T1} is positioned differently from that of the corresponding region in *E. coli* RecQ. The bacterial and human RecQ structures also display a difference in the β -hairpin structure that forms part of the WH fold, which is significantly longer in RECQ1^{T1}. Biochemical characterization of a RECQ1^{T1} β -hairpin Y564A mutant suggests that the β -hairpin in RECQ1 may play a critical role in DNA strand separation [42]. Overall, the structure of RECQ1^{T1} shares major structural features with *E. coli* RecQ; however, the subtle differences may be highly important for certain unique aspects of the catalytic activities of RECQ1. The DNA substrate specificity and ability of RECQ1 to efficiently catalyze branch migration and assume alternative conformational states for DNA unwinding or strand annealing are discussed below.

DNA Substrate Specificity of RECQ1 Helicase

RECQ1 is a 3' to 5' DNA helicase as determined by a conventional strand displacement assay with a DNA substrate that contains an intervening ssDNA between two duplexes within the same DNA substrate [44] or oligonucleotide-based substrates with 3' ssDNA overhangs [45]. For a detailed discussion of the DNA substrate specificity of RECQ1 helicase activity, please see [8,46]. For standard duplex DNA substrates, RECQ1 was shown to unwind (in order or preference) forked duplex, 3' overhang or 3'-flap, 5'-flap, and synthetic replication fork structures. Thus, RECQ1 unwinds conventional duplex DNA substrates representing key replication and repair intermediates that lack single-strand character in the 3', 5', or both arms adjacent to the duplex. Some more recent developments in this area will be discussed here.

RECQ1, like a number of other RecQ helicases (e.g., WRN, BLM, and Sgs1) unwinds a synthetic HJ structure to generate a splayed arm product in a reaction dependent on ATP hydrolysis [47]. The bacterial HJ recognition protein RuvA inhibits RECQ1 branch migration, suggesting that RECQ1 initiates unwinding at the junction crossover. The N-terminal region of RECQ1 is required for HJ unwinding and also protein oligomerization, suggesting that the N-terminal domain or higher order oligomer formation promoted by this region is necessary for RECQ1 to disrupt HJs [46]. If oligomerization is required for RECQ1 to unwind HJs, this scenario would be similar to that of WRN which was recently shown to unwind the HJ structure as a tetramer [48].

RECQ1 also unwinds the three-stranded D-loop structure [47]. D-loops can form during an early step in homologous recombination when a recombinase protein binds ssDNA and mediates invasion of the ssDNA molecule into a homologous duplex. RECQ1 unwinds D-loops with either a protruding single-stranded 3'- or 5'-tail by releasing the invading third strand from D-loop structures in an ATP- and protein concentration-dependent manner; however, preferential unwinding of the D-loop with a protruding single-stranded 3'-tail was observed over the entire range of RECQ1 protein concentrations.

Recently, two groups reported that RECQ1 fails to unwind G-quadruplex substrates under conditions that the helicase efficiently unwinds standard duplex DNA substrates [46,49]. The inability to resolve this particular form of alternate DNA structure distinguishes RECQ1 from WRN, BLM, Sgs1, or *E. coli* RecQ helicases which have the ability to efficiently unwind a variety of G-quadruplex DNA substrates [3]. A comparison of RECQ1 and BLM helicase substrate specificity revealed several other differences including the inability of RECQ1 to unwind a DNA-RNA hybrid, catalyze fork regression, or displace plasmid D-loops lacking a 3'-tail [46]. Curiously, RECQ1 but not BLM, was able to unwind four-armed synthetic HJ structures that lacked an homologous core [46].

Aside from helicase-catalyzed unwinding of DNA structures, certain RecQ helicases (BLM [50], RECQL5 [51]) can use their motor ATPase to inhibit Rad51-mediated strand exchange by stripping RAD51 from DNA, a function that is thought to be important for regulating homologous recombinational repair; however, RECQ1 is unable to do so [50]. RECQ1, WRN and BLM all fail to displace streptavidin from a biotinylated oligonucleotide under conditions that each helicase is active as a DNA unwinding enzyme [52]. In contrast, the SF2 helicase FANCD1 efficiently disrupts the high affinity biotin-streptavidin interaction, destabilizes the Rad51-ssDNA interaction, and also inhibits Rad51 strand exchange [52]. Presumably the differences in substrate specificity between RECQ1 and other SF2 helicases are important for their respective roles in cellular DNA metabolism.

RECQ1 Helicase Activity on Damaged DNA Substrates

The importance of RECQ1 for cell proliferation and an appropriate DNA damage response is apparent from cellular studies in mouse and human. Since the RecQ helicases have important roles in DNA repair and act as DNA translocases, it has been informative to characterize their ability to unwind damaged DNA molecules.

Effect of Benzopyrene Adduct on RECQ1 Helicase Activity—Benzo[*a*]pyrene (BaP) in the diet and air from combustion of fuel and tobacco form bulky DNA adducts that impede replication and induce mutations by causing replication errors. BaP DE-dG trans adducts occupy the minor groove, but do not significantly distort the double helix of B-form DNA [53-55]. RECQ1 helicase activity on the BaP DE-dG adducted substrate was inhibited in a strand-specific manner, i.e., when the adduct was positioned in the strand that RECQ1 is presumed to translocate based on its demonstrated 3' to 5' directionality of unwinding [56]. A possible mechanism for the profound inhibition of RECQ1 helicase activity by the minor groove BaP DE-dG adduct is suggested by a recently identified conserved helix-turn-helix motif found in RecQ helicases that was shown to mediate minor groove binding in the human DNA repair protein *O*⁶-alkylguanine-DNA alkyltransferase [57]. Further studies of minor groove modifications on RECQ1 helicase activity are warranted since this form of damage is a consequence of many different agents that induce genotoxic stress, including both noncovalent and covalent damage. For example, the WRN and BLM helicases were profoundly sensitive to helicase inhibition by the minor groove binders distamycin and netropsin [58].

Effect of Backbone Discontinuity on RECQ1 Helicase Activity—Structural data for Superfamily (SF) 2 helicases to which RECQ1 belongs suggest that this class of DNA unwinding enzymes interacts with nucleic acids by contacts with the phosphodiester backbone. In contrast, SF1 helicases bind nucleic acids through hydrophobic interactions with the bases [59]. For example, the ability of SF1 Pcr helicase to interact directly with bases is proposed to be important for the base flipping mechanism it uses to unwind duplex DNA [60]. RECQ1 helicase activity was tested on forked duplex substrates that harbored a synthetic polyglycol modification to the sugar phosphate backbone within the duplex region in either the non-translocating or translocating strand that the enzyme is presumed to translocate. The polyglycol modification spans 3 base pairs within duplex DNA or 6 nucleotides within unwound ssDNA. RECQ1 helicase was inhibited in a strand-specific manner such that the polyglycol modification in only the translocating strand blocked RECQ1 unwinding [61]. Endogenous alkyl phosphotriester lesions or other sugar phosphate backbone modifications that disrupt strand continuity are predicted to inhibit RECQ1 in a strand-specific manner. This may be distinct from those SF1 helicases that intimately interact with the bases during unwinding.

Effect of Thymine Glycol on RECQ1 Helicase Activity—Thymine glycol, a base lesion that results from ionizing radiation and other forms of oxidative stress [62], induces a significant localized structural change to DNA with the thymine glycol largely extrahelical

[63]. Thymine glycol is considered a lethal lesion as a consequence of its blocking effect on cellular DNA replication and transcription. RECQ1 was tested on a forked duplex substrate with a single thymine glycol in either the translocating or nontranslocating strand within the duplex region. Inhibition of RECQ1 helicase activity was observed only when the thymine glycol resided in the translocating strand [64]. The single-stranded DNA binding protein Replication Protein A (RPA), which physically interacts with RECQ1 and stimulates its unwinding activity on long undamaged DNA substrates [65], was tested for its ability to stimulate RECQ1 unwinding of the DNA substrate with the thymine glycol in the helicase translocating or non-translocating strand. RPA was unable to stimulate RECQ1 helicase activity on the DNA substrate with thymine glycol in the translocating strand; however, RPA stimulated RECQ1 helicase activity on the substrate with thymine glycol in the non-translocating strand [64]. Thus, RPA stimulates RECQ1 helicase activity in a strand-specific manner, *i.e.*, RPA stimulates DNA unwinding by RECQ1 when the thymine glycol is positioned in the non-translocating strand for the 3' to 5' RECQ1 helicase. Interestingly, RPA stimulated DNA unwinding of the FANCD1 5' to 3' helicase in a strand-specific manner as well, only when the thymine glycol was positioned in the opposite strand that FANCD1 translocates. The high affinity binding of RPA to ssDNA containing thymine glycol is likely to be a mechanistic component of the functional interaction between RPA and the SF2 helicases RECQ1 and FANCD1 [64]. A model was proposed in which RPA binds with high affinity to the exposed thymine glycol in the unwound ssDNA opposite to the strand the helicase is tracking along. The physical interaction of RPA with the helicase is also likely to be important since a heterologous ssDNA binding protein from *E. coli* failed to stimulate helicase-catalyzed DNA unwinding of DNA substrates harboring the thymine glycol base damage.

RECQ1 Strand Annealing and Assembly State Controlled by Nucleotide-induced Conformational Switch

RECQ1, like other DNA helicases and motor ATPases (Table 1), was found to catalyze strand annealing of complementary single-strand DNA molecules [47]. ATP binding to RECQ1 induces a conformational change in the protein that serves as a molecular switch from a strand-annealing to a DNA-unwinding mode [47] (Fig. 2). Different quaternary states of RECQ1, modulated by ssDNA and ATP binding, are associated with its strand annealing or DNA unwinding activities [66]. RECQ1 efficiently promotes strand annealing as a higher order oligomer (pentamer or hexamer) whereas smaller oligomeric states (dimer or monomer) act to unwind duplex DNA. Electron microscopy reconstructions of the higher order oligomeric form revealed a cage-like structure that forms a hollow channel (see [66] for details). It would be of interest to determine if the physical interaction of RECQ1 with other proteins confers a preference for one assembly state or another, thereby regulating its dual enzymatic activities. For example, RPA which physically interacts with RECQ1 and stimulates its helicase activity [45,65], may help RECQ1 to favor its smaller oligomeric state that efficiently unwinds DNA.

From a biological standpoint, it is very important to determine the significance of RECQ1 strand annealing and DNA unwinding activities. As discussed more extensively in [8], the coordinate action of DNA unwinding and annealing may play a role in fork regression or synthesis dependent strand annealing, a pathway of double strand break repair. The elevated SCEs and spontaneous γ -H2AX foci in RECQ1-deficient cells would be consistent with a role of RECQ1 in a pathway of homologous recombinational repair. Coordination between strand annealing and DNA unwinding would likely influence directional migration of a D-loop or HJ structure.

RECQ1 DNA Branch Migration Activity

RECQ1 was reported to promote ATP-dependent three-stranded and four-stranded DNA branch migration, showing greater efficiency in the three-stranded reaction [67]. RECQ1

strongly preferred to promote branch migration in the 3' to 5' direction for both three-stranded and four-stranded reactions. The unidirectional 3' to 5' polarity for branch migration distinguishes RECQ1 from BLM helicase and RAD54 which do not display significant preference in branch migration directionality [67]. Given that the N-terminal region of RECQ1 is required for Holliday junction unwinding and also protein oligomerization [46], it will be of interest to determine the assembly state of the RECQ1 species that promotes branch migration, and the functions of RECQ1 protein domains in DNA branch migration activity.

The ability of RECQ1 to promote branch migration with 3' to 5' polarity enables RECQ1 to disrupt three-stranded D-loop recombination intermediates arising from invasion of the protruding 5' ssDNA tail into the recipient duplex [67]. This particular form of the D-loop is inert to extension by a DNA polymerase which requires a base paired nucleotide with a free 3' hydroxyl. Therefore, the D-loop substrate efficiently branch-migrated by RECQ1 *in vitro* may represent a dead-end substrate intermediate of homologous recombination that arises during double strand break repair. It is proposed that through its branch migration activity, RECQ1 may prevent the accumulation of potentially toxic homologous recombination intermediates and facilitate the restart of collapsed replication forks.

RECQ1 Protein Interactions

From nuclear extract co-immunoprecipitation experiments and biochemical assays with purified recombinant proteins, RECQ1 was found to interact with several DNA repair factors that regulate genetic recombination. These include MSH2/6, MLH1-PMS2, EXO1 [68], and RAD51 [26]. RECQ1 stimulated the endonucleolytic and exonucleolytic incision activities of EXO1 through an ATP-independent protein interaction [68], suggesting that RECQ1 and EXO1 may function together in the resolution of DNA structural intermediates formed during DNA repair or replication; however, the biological significance of the protein interaction is not yet known.

It is conceivable that the increased sensitivity of mouse and human RECQ1-deficient cells to ionizing radiation [25,26] reflects a role of RECQ1 with structure-specific nucleases in end processing of strand breaks [69]. Recently, a number of studies from several laboratories have provided evidence that EXO1 plays an important role in end resection during double strand break repair. Exo1 and Sgs1, the sole RecQ homolog in *S. cerevisiae*, were shown to have a role in long range 5' strand resection at double strand breaks at a step after initial trimming by the MRX/Sae2 complex ([70-72]. Furthermore, Sgs1 is required in order for Dna2 to compensate for the loss of Mre11 nuclease during repair of IR-induced damage [73]. Using fission yeast as a model system, it was shown that in the absence of MRN, EXO1 becomes the major nuclease required for efficient meiotic recombination [74]. Recently, the Kowalczykowski lab demonstrated that the BLM helicase stimulates the nucleolytic activity of EXO1 on a linearized plasmid DNA molecule in an ATP-independent manner [75]. The earlier finding that WRN interacts physically and functionally with EXO1 [76] suggests that EXO1 may be a conserved protein partner of the RecQ helicases. Conceivably, the interaction of EXO1 with the various human RecQ helicases (RECQ1, WRN, BLM) is tailored for specific situations such as repair of strand breaks, restoration of blocked replication forks, or at specialized DNA structures such as telomeres.

Avenues for Investigation of RECQ1 Biological Function

RECQ1 polymorphism studies have suggested that RECQ1 may play a role in cancer [77, 78]. However, mutations in RECQ1 (or RECQ5 β) have not yet been directly linked to a genetic disease and the RECQ1 knockout mouse lacks organismal or tissue-specific phenotypes [25]. Therefore, understanding the role of RECQ1 in nucleic acid metabolism and suppression of pathological symptoms may be more elusive than other DNA helicases. We will focus this

portion of the review on some recent discoveries that lead us to propose research directions for understanding how RECQ1 is important (Fig. 3).

Does RECQ1 Have a Role at Telomeres?

Certain members of the RecQ helicase family have been implicated in telomere metabolism (for review, see [79]). *S. cerevisiae* Sgs1 plays a role in telomere metabolism [80-82]. In mammalian cells, both WRN and BLM helicases are also believed to be involved in telomere maintenance (for review, see [79]). However, FISH and flow cytometry analyses of mouse *Recql*^{+/+}, *Recql*^{+/-}, and *Recql*^{-/-} splenocytes and thymocytes did not reveal any differences in telomere length [25]. Expression levels of the known family members WRN, BLM, RECQL4, and RECQL5 were not altered in *Recql* knockout mice, suggesting that if one or more of these helicases compensates for the absence of RECQL, the normal level of expression is sufficient for this compensation. The genetic background and/or tissue specificity of RECQL function may protect the mouse from severe abnormalities that might be expected from the magnitude of chromosomal aberrations detected at the cellular level. For example, WRN-deficient mice lack a disease phenotype [83,84]; however, late-generation telomerase- and WRN-deficient mice (*mTerc*^{-/-} *Wrm*^{-/-}) displayed clinical symptoms of premature aging and the types of tumors typically observed in Werner syndrome patients [85,86]. It is plausible that the manifestation of disease phenotypes of RECQL deficiency in mice is related to telomere maintenance or some other event critical for genomic stability.

Genetic data from yeast implicate the sole RecQ homolog Sgs1 in telomere metabolism through a recombination mediated pathway known as Alternative Lengthening of Telomeres (ALT) [81,87]. A recent study using a de novo telomere addition assay showed that Sgs1 is likely required for Exo1 to act at telomeres in C strand degradation [88]. The authors provide further evidence that Sgs1 controls telomere length maintenance by regulating telomere processing.

The interaction of human RECQ1 with EXO1 [76] may also be important in telomere metabolism. EXO1 nuclease activity was shown to have a role in stability of chromosome ends (for review, see [89]). In a proposed model, EXO1 removes the lagging strand template strand to yield a 3' ssDNA overhang structure that can be properly processed to maintain telomere stability. As an auxiliary factor for EXO1, RECQ1 may facilitate the 5' end trimming function of EXO1. Using a procedure called Proteomics of Isolated Chromatin Segments (PICH) that isolates genomic DNA with its associated proteins, it was found that RECQ1 and other proteins were purified with human telomeric chromatin specifically in cells that use the ALT pathway [90]. It will be of interest to determine whether RECQ1 interacts with telomere factors such as the single-stranded DNA binding protein POT1 and the telomeric sequence specific binding protein TRF2 to which WRN interacts [91-93]. RECQ1 and EXO1 may collaborate in a complex to accurately process chromosome ends. This may be in addition to an interactive role of RECQ1 and EXO1 in double strand break repair.

Involvement of RECQ1 in Herpes Virus Lytic DNA Replication

RECQ1 depletion resulted in a significantly reduced DNA content compared to control HeLa cells [26]. Mitogenic efficiency was also reduced as evaluated by bromodeoxyuridine incorporation as a measurement of DNA synthesis. Therefore, RECQ1-depleted cells are impaired for their ability to synthesize DNA as compared to control siRNA treated cells. The impaired cell proliferation of RECQ1-deficient cells may reflect a direct role of RECQ1 in cellular DNA replication that is required during a specialized circumstance such as replicational stress that occurs from endogenous DNA damage.

Although RECQ1 has not been directly implicated in replication, evidence suggests that some of the other RecQ helicases have roles in replication, either during initiation or elongation.

Certain RecQ helicases (e.g., WRN [94]) are associated with a DNA replication complex isolated from mammalian cells. Although the mammalian RecQ helicases are not essential for replication since the corresponding mutant cell lines are viable, this does not exclude a more specialized role. Viral DNA replication can provide a window to the identification of nuclear protein factors in replication. A helicase deficiency can negatively affect viral DNA replication which relies heavily on the host cell machinery. For example, herpes simplex virus yield is reduced in WRN-deficient cells compared to control human fibroblast cells, and WRN is associated with the viral single-stranded DNA binding protein ICP8 [95].

Herpesvirus lytic DNA replication requires the cis-acting element, origin, and trans-acting factors, including virally encoded origin-binding protein, DNA replication enzymes, and auxiliary factors. Two lytic DNA replication origins (ori-Lyt) of Kaposi's sarcoma-associated herpesvirus (KSHV) have been identified, and two virally encoded proteins (RTA and K8) have been shown to bind to the origins [96]. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains two functional lytic origins of DNA replication [97]. Using a nuclear extract derived from the primary effusion lymphoma cell line BCBL-1 that carries latent KSHV and a biotin-labeled DNA fragment of KSHV ori-Lyt core domain as bait, Wang et al. (2008) employed a DNA affinity purification and mass spectrometry procedure to show that RECQ1 is physically associated with KSHV ori-Lyt through K8 and RTA [98]. RECQ1 was able to be co-immunoprecipitated by both K8 and RTA viral proteins, suggesting that RECQ1 is an integral component of the pre-replication complex. Interestingly, RECQ1 is the only cellular protein that was detected in the pre-replication complex, suggesting a unique function of RECQ1 in initiation of viral DNA replication.

More recently, it was found that RECQ1 is associated with the OriLyt and Zta of another virus, Epstein-Barr virus (EBV)[99]. Depletion of RECQ1 by shRNA resulted in reduced lytic DNA replication. Identification of RECQ1 in the KSHV and EBV replication complexes suggests that the cellular RECQ1 helicase may be involved in unwinding origin DNA during the initiation of KSHV and EBV lytic DNA replication. Future studies such as CHIP experiments to examine the association of RECQ1 with DNA replication factories at origins should be encouraged. Replication of certain regions of the genome may require a RecQ helicase for replication initiation (RECQ4, [100]) or normal fork progression during replicational stress (WRN, [101]).

RECQ1 and Gene Regulation

Genotoxic stress in the form of DNA damage can lead to mutations which predispose individuals to cancer. Cellular senescence might also be enhanced by DNA damage which can suppress tumor formation [102]. A growing field of interest is how persistent DNA damage triggers senescence-associated phenotypes. Cellular studies demonstrating increased DNA damage load in RECQ1-deficient cells suggests that RECQ1, the most abundant of the RecQ helicases, plays an important role in the DNA damage response. One hypothesis is that RECQ1 plays a role in the regulation of gene expression as a component of the DNA damage response. Currently, we have undertaken a microarray analyses of gene expression patterns in primary mouse embryonic fibroblasts (MEFs) from RECQ1 knockout and wild-type mice. MEFs were exposed to a variety of DNA damaging agents and allowed to recover for short or long time periods. RNA was then collected and examined by microarray analyses to determine if differences between RECQ1 wild-type and knockout MEFs exist, and if there are stress-specific gene expression changes. Our unpublished results indicate that RECQ1 knockout MEFs show gene expression changes as a function of the DNA damage treatment that are clearly different from those of MEFs from the wild-type littermates. Further studies in this area are underway, leading us to believe that RECQ1 status influences the gene expression response after various forms of DNA damage (Wu *et al.*, manuscript in preparation).

Summary

In this review, we have attempted to provide an up to date summary of the research pertaining to RECQ1. In many areas, this research lags behind that of the other RecQ helicases. However, certain recent advances including the genetic characterization of RECQ1 in model systems and the demonstration of cellular phenotypes in RECQ1-deficient mammalian cells suggest a unique role of RECQ1 in cellular DNA metabolism. A linkage to disease or cancer may await the future of RECQ1 research, in which case ongoing studies in basic research to provide a molecular understanding of RECQ1 functions and pathways will likely aid in understanding the basis for phenotypes associated with RECQ1 deficiency.

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Fig. 1A

Fig. 1B **Fig. 1. Alignment and phylogenetic tree of RECQ1-like helicases across species**

Panel A, The conserved helicase core domain is indicated by yellow, the RecQ C-terminal domain (RQC) in purple, and the Helicase and RNase D C-terminal domain (HRDC) in green. *Panel B*, The amino acid sequences of the full length proteins in *Panel A* were aligned and a phylogenetic tree was constructed by ClustalX2 with number of bootstrap trials at 1000. The image was generated in Treeview. Branch numbers refer to bootstrap values.

Fig. 2. Nucleotide-induced conformational switch controls RECQ1 helicase and strand annealing activities

A model is presented depicting the importance of ATP-induced conformational change in RECQ1 for regulation of its catalytic activity as a DNA helicase or strand annealing protein. Assembly state of RECQ1 in terms of oligomerization is not depicted in the model.

Fig. 3. Potential cellular functions of RECQ1 in DNA metabolism
See text for details.

Table 1
DNA Helicases and Motor ATPases that Catalyze Strand Annealing

Proteins		References
Human	RECQ1	[47,66]
	BLM	[103,104]
	RECQ4	[104,105]
	WRN	[104,106]
	RECQ5 β	[107]
	DNA2	[108]
	CSB	[109]
	PIF1	[110]
	HARP	[111]
	RAD54	Personal communication, A. Mazin
Yeast	DNA2	[108]
Drosophila	BLM	[104,112]
Bacteriophage	UVsW (T4)	[113]
Archea	Hjm/Hel308A (<i>S. tokodaii</i>)	[114]
	Hel112 (<i>S. solfataricus</i>)	[115]