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Human RECQL5: Guarding the crossroads of DNA replication and transcription and providing backup capability

Venkateswarlu Popuri, Takashi Tadokoro, Deborah L. Croteau, and Vilhelm A. Bohr

Laboratory of Molecular Gerontology, National Institute on Aging, NIH, Baltimore, MD, USA

Abstract

DNA helicases are ubiquitous enzymes that catalyze unwinding of duplex DNA and function in all metabolic processes in which access to single-stranded DNA is required, including DNA replication, repair, recombination and RNA transcription. RecQ helicases are a conserved family of DNA helicases that display highly specialized and vital roles in the maintenance of genome stability. Mutations in three of the five human RecQ helicases, BLM, WRN and RECQL4 are associated with the genetic disorders Bloom syndrome, Werner syndrome and Rothmund-Thomson syndrome that are characterized by chromosomal instability, premature aging and predisposition to cancer. The biological role of human RECQL5 is only partially understood and RECQL5 has not yet been associated with any human disease. Illegitimate recombination and replication stress are hallmarks of human cancers and common instigators for genomic instability and cell death. *Recql5* knockout mice are cancer prone and show increased chromosomal instability. *Recql5*-deficient mouse embryonic fibroblasts are sensitive to camptothecin and display elevated levels of sister chromatid exchanges. Unlike other human RecQ helicases, RECQL5 is recruited to single-stranded DNA breaks and is also proposed to play an essential role in RNA transcription. Here, we review the established roles of RECQL5 at the cross roads of DNA replication, recombination and transcription, and propose that human RECQL5 provides important backup functions in the absence of other DNA helicases.

Keywords

Base excision repair; DNA replication; homologous recombination; RecQ helicases; transcription

Biochemical and cellular characterization of human RECQL5

RecQ helicases play several critical functions in promoting genomic stability and are often called “caretakers of the genome” (Bohr, 2008; Chu & Hickson, 2009). Human cells express five RecQ helicases, while lower organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* express only one member per species. RECQL5 was first identified and cloned from human cells in 1998, based on its homology with the other human RecQ homologs (Kitao *et al.*, 1998). Unlike other RecQ helicases, RECQL5 exists in three alternatively-

Address for correspondence: Vilhelm A. Bohr, Laboratory of Molecular Gerontology, National Institute on Aging, NIH, 251 Bayview Blvd, Suite 100, Baltimore, MD 21224, USA. Tel: 410 558 8162. Fax: 410 558 8157. vbohr@nih.gov.

Declaration of interest

The authors declare no conflict of interest.

spliced isoforms, RECQL5 α , RECQL5 β and RECQL5 γ (Shimamoto *et al.*, 2000). RECQL5 α and RECQL5 γ are cytoplasmic in localization, and contain 410 and 435 amino acids, respectively (Figure 1). RECQL5 β is nuclear and contains 991 amino acids including an extended C-terminal region that is not homologous to that of the other human RecQ homologs (Figure 1) (Shimamoto *et al.*, 2000). RECQL5 α is truncated upstream of the putative Zn²⁺-binding motif of RecQ C-terminal domain, whereas RECQL5 γ contains 25 additional amino acids at the C-terminus that are not present in RECQL5 α and RECQL5 β (Figure 1).

All three RECQL5 isoforms contain the conserved helicase domain; however, only RECQL5 β possesses ATPase and/or helicase activity (Ren *et al.*, 2008). RECQL5 β can unwind forked duplexes and M13mpl8-based partial duplex DNA structures, whereas it does not efficiently unwind D-loop, G-quadruplex and Holliday junction (HJ) substrates (Garcia *et al.*, 2004; Ghosh *et al.*, 2009) that are preferentially unwound by BLM and WRN. RECQL5 β requires the single-stranded DNA binding protein Replication protein A (RPA) for efficient DNA unwinding activity, while RPA binding inhibits its strand annealing activity (Garcia *et al.*, 2004). It should also be noted that RECQL5 β lacks the winged-helix motif that plays a critical role in DNA unwinding activity of RECQL1 and WRN (Pike *et al.*, 2009; Tadokoro *et al.*, 2012). RECQL5 α possesses neither ATPase nor DNA unwinding activities, but surprisingly displays a strong strand annealing activity (Ren *et al.*, 2008). Although there are no reported studies on RECQL5 γ , its enzymatic properties may be similar to that of the α isoform. It is not known if RECQL5 α and RECQL5 γ isoforms possess any redundant roles with that of the relatively well characterized RECQL5 β . The larger RECQL5 β isoform is significantly expressed at a higher level than the other isoforms in both mice and humans and is the focus of this review and will be referred to as RECQL5 in the remainder of the text.

The relative expression of human RecQ helicases in different tissues and their modulation at different stages of the cell cycle in replicating cells helps us discern a putative biological function for RECQL5. While the expression of BLM, WRN and RECQL4 appears to be tissue specific, RECQL5 is expressed ubiquitously in all tissues, independent of their cell cycle phase (Kawabe *et al.*, 2000; Kitao *et al.*, 1998). Further, agents that stimulate B-cell transformation or proliferation cause significant up regulation of all human RecQ helicases except RECQL5 (Kawabe *et al.*, 2000). These observations indicate a general and cell cycle-independent role for RECQL5 and a possible role in G₀ and G₁ in resting cells, which is also compatible with its recent implications in transcription. RECQL5 differs from other human RecQ helicases in its possible role as a transcriptional modulator (Aygun *et al.*, 2009) and in its recruitment to single-strand breaks (SSBs) (Tadokoro *et al.*, 2012). Although not associated with any disease, RECQL5 was proposed to be a tumor suppressor and *Recql5* deletion in mice results in higher cancer susceptibility (Hu *et al.*, 2007). Hence, it is important to understand its biological role in the maintenance of genomic stability.

RCQ-5, the RECQL5 homologue in *Caenorhabditis elegans*, is expressed in all developmental phases and its perturbation reduced the life span of the organism and slightly increased its sensitivity to ionizing radiation (Jeong *et al.*, 2003). Genetic studies with *Drosophila melanogaster* RECQ5/QE, the closest fly homologue of human RECQL5, also

provided some insights into RECQL5 function in genome stability (Jeong *et al.*, 2000; Nakayama *et al.*, 2004, 2006). Flies carrying homozygous deletion of the RECQ5/QE gene are viable and fertile but exhibit mitotic defects and chromosomal abnormalities in larval neuroblasts (Nakayama *et al.*, 2009). Interestingly, RECQ5/QE was able to rescue several phenotypes such as higher frequencies of homologous recombination (HR), sister chromatid exchanges (SCEs), and increased sensitivity to methyl methanesulfonate (MMS) and hydroxyurea (HU) in the cells lacking the single budding yeast RecQ helicase Sgs1 (Nakayama *et al.*, 2004). Moreover, RECQ5/QE also complements the growth defects in *sgs1*, *srs2* and *sgs1 top3* yeast, suggesting a possible role in HR (Nakayama *et al.*, 2004). *Recql5*-deficient mice exhibit elevated frequencies of SCEs and chromosomal translocations in response to replicative stress (Hu *et al.*, 2007).

At least 10 human proteins have been shown to interact with RECQL5, as summarized in Table 1. These protein interacting partners suggest the involvement of RECQL5 in various DNA metabolic pathways including DNA replication, DNA double strand break (DSB) repair, base excision repair (BER) and transcription. Cellular studies are consistent with this notion, and the functional significance of RECQL5 protein interactions will be discussed in more detail in the following sections.

Role of RECQL5 in DNA replication and chromosomal segregation

Recent studies suggest that RECQL5 plays a role in DNA replication. *Recql5*-deficient mouse embryonic stem (ES) cells and mouse embryonic fibroblasts (MEFs) are hypersensitive to camptothecin (CPT), a drug that inhibits Topoisomerase I and blocks DNA replication (Hu *et al.*, 2009). Specifically, a higher proportion of *Recql5*-deficient MEFs failed to incorporate bromodeoxyuridine when compared to their wild-type counterparts after treatment with CPT. Moreover, the loss of replication capability upon CPT treatment was also accompanied by a rapid increase in DSB accumulation, gross chromosomal rearrangements and replication-dependent cell death (Hu *et al.*, 2009). Co-treatment with CPT and aphidicolin, an inhibitor of DNA replication (Ishii & Bender, 1980), can alleviate the cytotoxic effects in both wild-type and *Recql5*-deficient ES cells, suggesting that the enhanced cytotoxicity of CPT in *Recql5*-deficient cells is in part due to a defect in DNA replication (Hu *et al.*, 2009). The amino acid composition of the mouse and human RECQL5 proteins is 78% identical (Kawabe *et al.*, 2000; Shimamoto *et al.*, 2000) and expression of human RECQL5 in *Recql5*^{-/-} ES cells can partially rescue sensitivity to CPT, indicating that the function of RECQL5 is partially conserved in mammals (Hu *et al.*, 2009).

Mouse *Recql5*^{-/-} ES cells also show an increase in spontaneous γ H2AX foci that are positive for proliferating cell nuclear antigen (PCNA), indicating that these DNA lesions might be associated with DNA replication (Hu *et al.*, 2007). Spontaneous DNA strand breaks also accumulate in *D. melanogaster* RECQ5/QE mutant larvae (Nakayama *et al.*, 2009) and in human RECQL5 knockdown cells (Li *et al.*, 2011). Such an elevated level of DSBs might not be associated with a defect in DSB repair, as these cells are proficient in HR (Hu *et al.*, 2007, 2009). However, it is possible that RECQL5 plays an important role in maintaining active DNA replication to prevent replication fork collapse and generation of DSBs. This implies possible roles during normal DNA replication and also in cells

experiencing replicative stress. For instance, RECQL5 physically interacts with human Flap Endonuclease 1 (FEN1), which plays a critical role in the processing of Okazaki fragments during DNA replication and dramatically stimulates the rate of FEN1 cleavage on both 5' flap and nicked DNA substrates (Speina *et al.*, 2010). This raises the intriguing possibility that RECQL5, via stimulation of FEN1, could also coordinate the cleavage event needed to reset an uncoupled replication fork (Speina *et al.*, 2010). Furthermore, RECQL5 can promote strand exchange on synthetic DNA structures that resemble a stalled replication fork. In particular, RECQL5 can overcome a leading strand gap and can promote the displacement of both arms of the fork and subsequently anneal the displaced leading and lagging strands, suggesting that it can mediate regression of stalled replication forks to facilitate bypass of DNA damage via template-switching (Kanagaraj *et al.*, 2006). RECQL5 associates with DNA replication factories in S phase nuclei, persists at the sites of stalled replication forks, and physically interacts with PCNA both *in vivo* and *in vitro* (Kanagaraj *et al.*, 2006). In thymidine-treated cells, RECQL5 relocates to stalled replication forks, suppresses thymidine-induced RPA foci, and inhibits CHK1 signaling and γ H2AX activation and has been proposed to be involved in replication fork stabilization (Blundred *et al.*, 2010). Furthermore, the amount of chromatin-bound RECQL5 co-localizing with CldU also increases in thymidine- and HU-treated cells (Blundred *et al.*, 2010).

It was reported that CPT-induced Topo I–DNA complex formation can result in retardation of DNA supercoiling which is also associated with an impediment of transcription and replication *in vivo* (Koster *et al.*, 2007). This may also suggest a possible involvement of RECQL5 in DNA super-coiling and in DNA decatenation and/or chromosomal separation. Our recent observations indicate a physical and functional interaction of Topoisomerase II α with RECQL5 and this direct interaction stimulates the DNA decatenation activity of Topoisomerase II α (Ramamoorthy *et al.*, 2012). Stable depletion of RECQL5 compromises cell proliferation, induces late S-phase cycling defects in cells and activates a G2/M decatenation checkpoint leading to apoptosis (Ramamoorthy *et al.*, 2012). These effects are similar to those observed after depletion of Topoisomerase II α (Bower *et al.*, 2010; Gonzalez *et al.*, 2011). Moreover, stable RECQL5 knockdown cells are also characterized by the presence of under-condensed and entangled chromosomes (Ramamoorthy *et al.*, 2012). In separate studies, loss of maternal RECQL5 in *Drosophila* embryos was also reported to induce formation of DNA bridge-like structures in anaphase chromosomes, indicating chromosomal lagging or defects in chromosomal segregation (Sakurai *et al.*, 2011). Together, these observations link RECQL5 with topoisomerases and suggest that it participates in chromosomal segregation.

Role of RECQL5 in recombination and DNA repair

Role of RECQL5 in HR

In mice, *Recql5*-deficiency leads to genome instability (Hu *et al.*, 2007, 2009). Specifically, *Recql5*^{-/-} ES cells and primary MEFs show a high level of SCEs and an increased rate of chromosomal rearrangements, an end result of RAD51-dependent HR-mediated DSB repair. The elevated SCE frequency in *Recql5*^{-/-} cells is reminiscent of the phenotype of BLM deficient cells, suggesting that RECQL5 and BLM share an anti-recombination function (Hu

et al., 2005). Indeed, both BLM and RECQL5 can dissociate RAD51 from ssDNA *in vitro* (Bugreev *et al.*, 2007, 2009; Hu *et al.*, 2007). Mouse *Recql5*^{-/-} *Blm*^{-/-} double mutant cells exhibit an even greater SCE frequency than the *Recql5*^{-/-} or *Blm*^{-/-} single mutant cells (Hu *et al.*, 2005), suggesting non-redundant roles for RECQL5 and BLM in suppressing SCEs. Interestingly, RECQL5 interacts with topoisomerase IIIα (Shimamoto *et al.*, 2000), but does not substitute for BLM in double HJ dissolution (Raynard *et al.*, 2006; Wu *et al.*, 2005).

A physical interaction between RECQL5 and RAD51 is essential for the anti-recombinase activity of RECQL5 *in vitro* (Schwendener *et al.*, 2010). RECQL5 contains a single RAD51-interacting domain located between amino acids 654 and 725 (Schwendener *et al.*, 2010). Deletion of amino acids 652–674 or alanine substitution of F666 (F666A) completely abolished RECQL5 and RAD51 binding and inhibited the anti-recombinase activity of RECQL5. RECQL5 contains a BRC variant motif (BRCv) in the RAD51 binding domain, similar to the BRC motif in BRCA2, and was shown to regulate its anti-recombinase activity *in vitro* (Islam *et al.*, 2012). Mutations in the BRCv repeat disrupts the major functions of RECQL5, association with RAD51 and its ability to block RAD51-mediated D-loop formation, suppression of SCEs, and resistance to CPT-induced replication stress (Islam *et al.*, 2012). In this context, it would be interesting to evaluate any functional interactions and/or involvement of RECQL5 in the BRCA2-mediated recombination pathway.

Role of RECQL5 in DSB repair

Our recent studies indicate that RECQL5-depleted cells accumulate spontaneous DNA DSBs and exhibit slower DNA repair capacity followed by γ -irradiation (Popuri *et al.*, 2012). We and others have shown direct recruitment of RECQL5 to DSBs (Popuri *et al.*, 2012; Zheng *et al.*, 2009) and constitutive association between RECQL5 and the MRE11-RAD50-NBS1 (MRN) complex (Zheng *et al.*, 2009), a primary sensor of DSBs. The MRN complex promotes DSB repair and regulates DNA damage signaling via activation of ATM kinase. RECQL5 interacts with the MRN complex through the MRE11 and NBS1 subunits and specifically inhibits MRE11 exonuclease activity (Zheng *et al.*, 2009). RECQL5 co-localizes with the MRN complex in cells exposed to HU or ultraviolet light (UVC), both of which stall replication fork progression (Zheng *et al.*, 2009). More importantly, MRE11 plays an essential role in recruiting RECQL5 to laser-induced DNA DSBs and other types of DNA damage associated with replication arrest (Zheng *et al.*, 2009).

In living cells, visualized using confocal laser microscopy, RECQL5 is recruited to DNA DSBs with similar kinetics as other human RecQ helicases such as BLM, WRN and RECQL4 (Popuri *et al.*, 2012). However, RECQL5 remains at DSBs for a shorter period of time than BLM and WRN but for a longer period of time than RECQL4 (Popuri *et al.*, 2012). These studies might reflect the differential involvement of RecQ helicases in DNA DSB repair. Mapping of domains within RECQL5 that are necessary for recruitment to DSBs revealed that both the helicase and KIX domains of RECQL5 are required for DNA damage recognition and for stable association to the DSB sites (Popuri *et al.*, 2012).

Although both these domains are recruited independently, they disassociate faster than the full length RECQL5 protein, indicating that RECQL5 requires both domains in order to be retained efficiently at DSBs and to facilitate efficient DNA repair (Popuri *et al.*, 2012). In a

separate study, Islam *et al.* showed that RECQL5 requires both the helicase and KIX domains to correct SCEs and to confer cellular resistance towards CPT (Islam *et al.*, 2010).

Consistent with its role in DSB repair, deletion of RCQ-5 in *Caenorhabditis elegans* also results in germ line sensitivity to ionizing radiation (Jeong *et al.*, 2003). *Drosophila* RECQ5/QE is also proposed to be involved in DSB repair and interestingly, the protein is up-regulated in *drosophila* S2 cells exposed to MMS (Nakayama *et al.*, 2006). Loss of function of RECQ5/QE leads to genome instability and loss of heterozygosity (Chen *et al.*, 2010). RECQL5 was also proposed to function in the resolution of knot-like DNA structures during mitosis and can play an important role in DSB repair during interphase in syncytial *Drosophila* embryos (Nakayama *et al.*, 2009). Loss of maternally derived RECQ5/QE leads to accumulation of spontaneous DSBs during mitosis in syncytial embryos (Nakayama *et al.*, 2009).

Role of RECQL5 in single-strand break repair and BER

BER is the most prominent pathway for repair of endogenous DNA damage. Endogenous reactive oxygen species and exogenous xenobiotic compounds can induce DNA base damages. SSBs generated by exogenous DNA-damaging agents or during processing of other types of DNA damage can also be repaired by BER. SSB repair (SSBR) requires several components of the BER pathway such as FEN1, PCNA and poly(ADP-ribose) polymerase 1 (PARP1). Since both FEN1 and PCNA form part of a core complex shared in replication and in BER and SSBR pathways (especially long patch BER sub-pathway), the interaction of RECQL5 with these proteins (Kanagaraj *et al.*, 2010; Speina *et al.*, 2010) implicates RECQL5 in these processes. Consistent with this hypothesis, our recent studies suggest that RECQL5 is involved in the repair of endogenous DNA damage (Tadokoro *et al.*, 2012). Specifically, the dissociation kinetics of XRCC1 foci was delayed in RECQL5-knockdown cells, suggesting inefficient SSBR in these cells (Tadokoro *et al.*, 2012). A large load of unrepaired SSBs contributes to genetic instability and can lead to accumulation of DNA DSBs and eventually cell death (Caldecott, 2008; Heeres & Hergenrother, 2007). Depletion of RECQL5 in human cells sensitizes them to oxidative stress, leads to accumulation of endogenous DNA damage and increases cellular PAR levels (Tadokoro *et al.*, 2012). In particular, DNA strand breaks and alkaline/Fpg sensitive sites, including Fapy and 8-oxo-dG, as well as abasic sites are more abundant in RECQL5-knock-down cells than in control cells (Tadokoro *et al.*, 2012). Consistent with these results, depletion of RECQ5/QE in *Drosophila* also results in the accumulation of DNA strand breaks, including SSBs and DSBs (Nakayama *et al.*, 2009). Moreover, *Recql5* deletion in mouse cells resulted in elevated SCEs, a phenomenon frequently associated with increased SSBs (Hu *et al.*, 2009). Involvement of RECQL5 in the BER and/or SSBR pathway is not unique among RecQ helicases, as BLM, WRN, RECQL4 and RECQL1 are all proposed to be involved in this pathway (Harrigan *et al.*, 2006; Schurman *et al.*, 2009; Sharma *et al.*, 2012; Szekely *et al.*, 2005). However, in contrast to the other human RecQ helicases, only RECQL5 accumulates at laser-induced DNA SSBs, suggesting a unique role of RECQL5 in this pathway (Singh *et al.*, 2010; Tadokoro *et al.*, 2012). Interestingly, *XRCC1* and *PARP1* are expressed at a lower level in RECQL5-depleted cells, suggesting that RECQL5 might have a possible role as a transcription modulator in relation to BER/SSBR genes (Tadokoro *et al.*,

2012). Collectively, these observations indicate that RECQL5 could play a non-redundant and unique role in BER, specifically in modulating and/or directly participating in BER-mediated repair of endogenous DNA damage.

Role of RECQL5 in transcription

A number of recent proteomic studies have revealed that RECQL5 forms a stable complex with RNA polymerase II (RNA Pol II) in human cells (Aygun *et al.*, 2008; Islam *et al.*, 2010; Kanagaraj *et al.*, 2010). The RECQL5–RNA Pol II interaction is direct and is mediated by the largest subunit of RNA Pol II, RPB1 (Aygun *et al.*, 2008). Knockdown of RECQL5 in human cells increases transcription of several genes (Izumikawa *et al.*, 2008). Further, *in vitro* transcription assays and small interfering RNA (siRNA) studies have shown that the RECQL5 inhibits RNA Pol II-catalyzed transcriptional initiation and elongation (Aygun *et al.*, 2009; Izumikawa *et al.*, 2008), while other RecQ helicases do not (Aygun *et al.*, 2008; Islam *et al.*, 2010), suggesting a specific role for RECQL5 in RNA metabolism. In agreement with this, several biochemical and bioinformatic analyses identified two regions in RECQL5 that could interact with RNA Pol II (Islam *et al.*, 2010): aa 540 to 620, homologous to the KIX domain in several Pol II transcriptional regulators (Parker *et al.*, 1996); and aa 909 to 991, homologous to the SRI (Set2 Rpb 1-interacting) domain in histone methyltransferase SetD2, which also regulates transcription (Kizer *et al.*, 2005). Interestingly, the SetD2-SRI domain interacts with the phosphorylated C-terminal domain (CTD) (pCTD) of Pol IIO, which is in complete agreement with the function of the predicted SRI domain in RECQL5 (Islam *et al.*, 2010; Li *et al.*, 2011). RECQL5 also co-purifies with subunits of the RNA Pol II-associated initiation complex (MED4, MED1, MED24 and MED 15) as well as with the elongation factors SPT5 and SPT6, indicating that RECQL5 likely plays an important role in the initiation and elongation phases of RNA Pol II-dependent transcription (reviewed in Aygun & Svejstrup, 2010).

Several studies have been carried out to elucidate the role of RECQL5 in transcription. It was proposed that RECQL5 associates with hypophosphorylated RNA Pol II, involved in transcription initiation (Pol IIA, via the KIX domain) and with hyperphosphorylated RNA Pol II, involved in the productive/ elongation phase of transcription (Pol IIO, via the SRI domain) (Islam *et al.*, 2010). It was speculated that RECQL5 might regulate RNA Pol II association with chromatin and possibly prevent spontaneous DNA breaks (Li *et al.*, 2011). A significant increase in the levels of chromatin bound RNA Pol II was found in cells lacking RECQL5 and more importantly, increased numbers of spontaneous DNA breaks were associated with RNA Pol II-dependent transcribed loci (Li *et al.*, 2011), indicating that the DSBs were transcription-associated. Furthermore, overexpression of RECQL5 in human 293 T cells reduced the amount of the elongation subunit RNA Pol IIO associated with DNA. In this context, the SRI domain of RECQL5 prevents the accumulation of RNA Pol IIO on chromatin, while overexpression of a RECQL5 fragment lacking the SRI domain in turn leads to an accumulation of the transcriptionally active form of RNA Pol II on chromatin and induces a p53-dependent transcriptional stress response (Li *et al.*, 2011). Collectively, these observations suggest that RECQL5 negatively regulates elongation by RNA Pol II *in vivo*. Interestingly, our previous observations indicate that WRN could be a potential transcriptional activator and can stimulate RNA Pol II-dependent transcription *in*

vitro (Balajee *et al.*, 1999). Consistently, Werner syndrome cells exhibit reduced levels of RNA Pol II-mediated transcription (Balajee *et al.*, 1999). These observations indicate differential involvement of these RecQ helicases in transcription.

Chromatin-remodeling factors also play important roles during transcription and can reportedly enhance the recruitment of RNA polymerase to the promoter-proximal region immediately upstream of the transcription start site, which is a prerequisite for the establishment of transcriptional machinery and the formation of the pre-initiation complex (PIC) (Thomas & Chiang, 2006). Chromatin modulators, such as the SWI/SNF chromatin-remodeling complex facilitate assembly of a bulky PIC complex at a promoter site (Li *et al.*, 2007; Venters & Pugh, 2009). In addition, chromatin-remodeling factors play important roles in other aspects of RNA Pol II transcription, such as transcription elongation and/or pre-mRNA processing (Li *et al.*, 2007). Recent mass spectrometry analyses demonstrate that the SWI/SNF complex (BRG1, BAF170, SNF5) interacts with RECQL5, providing a clue for the presence of RECQL5 in the SWI/SNF-RNAPII complex probably during the assembly of the PIC complex (Zhou *et al.*, 2010). In this context, RECQL5 could affect the recruitment of SWI/SNF chromatin-remodeling complex, thereby modulating assembly of the RNA Pol II transcription machinery.

RECQL5 safeguards the intersection of DNA replication and transcription

While RECQL5 may have separate roles in transcription and replication, recent studies link its potential involvement in regulating RNA Pol II with its role in suppressing DSBs during DNA replication. Interestingly, the interaction of RECQL5 with RNA Pol II was reportedly enhanced during S-phase and this increase may be required for regulating the levels of RNA Pol II molecules on DNA during S-phase (Li *et al.*, 2011). In RECQL5-depleted cells, spontaneous DSBs accumulate during replication, but only in association with RNA Pol II transcription (Li *et al.*, 2011). Transient incubation with the transcription inhibitor 5,6-dichloro-beta-D-ribofuranosyl benzimidazole (DRB) not only eliminated DNA-bound RNA Pol II, but also inhibited DNA strand breaks and γ H2AX foci in RECQL5-depleted cells (Li *et al.*, 2011). Even though transcription is required for cell viability, this essential cellular process is known to trigger DNA lesions in various organisms (Aguilera, 2002; Azvolinsky *et al.*, 2009; Gottipati & Helleday, 2009). Current models suggest that transcription-associated genome instability is intimately linked to DNA replication (Azvolinsky *et al.*, 2009; Gottipati & Helleday, 2009; Tuduri *et al.*, 2009). For example, a head-on collision between the transcription machinery and a replication fork is likely to cause fork stalling and/or collapse, leading to accumulation of spontaneous DSBs (Figure 2). RNA polymerase and the DNA replisome share the same DNA template for their respective functions. The rate of replication is typically 20 times faster than transcription and six or more replication forks may be present at the same time on the chromosome (Gotta *et al.*, 1991; Hirose *et al.*, 1983). Interference between replication and transcription represents a major source of spontaneous replication stress, which could drive genomic instability during the early stages of tumorigenesis (Tuduri *et al.*, 2009). Recent studies indicate that collisions of the transcription machinery with a replication fork are inevitable on long human genes and can result in the formation of fragile sites (Helmrich *et al.*, 2011). Hence, adaptation of the molecular machinery is absolutely necessary for the resolution of conflicts between

transcription and replication to ensure genomic stability. Unlike other human RecQ helicases, RECQL5 stands out uniquely with its strong inherent association with RNA Pol II and participates at the intersection of DNA replication and transcription. It has been proposed that RECQL5 promotes genome stabilization through two mechanisms: (1) by interacting with RNA polymerase IIa (via the KIX domain) and (2) via its catalytic/helicase activity (Islam *et al.*, 2010). Consistent with this notion, we observed that both the KIX and the helicase domain of RECQL5 are required for its recruitment and stable retention at DNA DSB sites (Popuri *et al.*, 2012). RECQL5 may inhibit Pol II transcription elongation to reduce the frequency of head-on collisions between transcription and replication machineries that would lead to stalled/collapsed replication forks (Rudolph *et al.*, 2007). RECQL5 may then directly stabilize and restart blocked replication forks (Kanagaraj *et al.*, 2006) by its catalytic activity (Figure 2).

Transcription can also enhance recombination events in all organisms ranging from prokaryotes to higher eukaryotes (Aguilera, 2002), which is a ubiquitous phenomenon referred to as transcription-associated recombination (TAR). The collision between transcription and replication machineries may result in replication fork stalling and recombination is the major mechanism involved in resolving the stalled forks (Figure 2) (Aguilera & Gomez-Gonzalez, 2008; Gottipati *et al.*, 2008). TAR may involve transient RNA: DNA hybrids (R-loops) that would also impair replication fork progression (Aguilera & Gomez-Gonzalez, 2008). RECQL5 cannot disrupt R-loops, but interestingly, depletion of RECQL5 reduces the cellular sensitivity to diospyrin, a plant-derived bisnaphthoquinonoid, which interferes with spliceosome assembly that also leads to the formation of R-loops (Kanagaraj *et al.*, 2010). These data suggest that RECQL5 negatively affects cell viability under conditions leading to the formation of R-loops. The ability of RECQL5 to regulate recombination, replication and transcription makes it a crucial guardian of genome integrity.

Interestingly, our preliminary observations indicate that depletion of RECQL5 may have more deleterious consequences in primary fibroblasts than in Simian virus (SV40)-transformed fibroblasts (Popuri *et al.*, 2012). This could be because SV40 T-antigen can activate transcription from certain RNA polymerase II and III promoters (Contreras & Fiers, 1981; Loeken *et al.*, 1988; Rajan *et al.*, 1991). It would be interesting in future studies to explore the role of RECQL5 in primary cells in comparison with the transformed cells. Consistent to our observations, it has been previously speculated that RECQL5 would have a critical function in resting/primary cells than the transformed cells based on its expression levels and due to its involvement in transcription (Kawabe *et al.*, 2000).

RECQL5 provides essential backup functions

Recent studies indicate that both BLM and RECQL5 are important anti-recombination factors, similar to Srs2 in yeast that blocks invasion of RAD51-ssDNA filaments onto duplex DNA to form D-loops (Bugreev *et al.*, 2007; Burgess *et al.*, 2009; Hu *et al.*, 2007). Mouse *Recql5*^{-/-} ES cells have an elevated frequency of SCEs and an increased incidence of multi-radial structures, similar to *Blm*^{-/-} cells (Hu *et al.*, 2005), but the overall frequency of SCEs in double-knockout ES cells (*Recql5*^{-/-} and *Blm*^{-/-}) is significantly higher than in *Blm*^{-/-} or *Recql5*^{-/-} ES cells (Hu *et al.*, 2005). Similar studies from Wang *et al.* (2003)

indicate that double depletion of RECQL5 and BLM in chicken DT40 cells results in a higher frequency of SCEs than in BLM^{-/-} cells, indicating that RECQL5 suppresses SCEs in the absence of BLM. Specifically, it is possible that RECQL5, perhaps in conjunction with BLM, plays an early role in preventing premature or unnecessary engagement of HR-mediated restart of stalled/ collapsed replication forks and this engagement could be more significant in the absence of BLM (Lu *et al.*, 2011). In this context, our studies indicate that RECQL5 is retained longer at DSB sites in BLM-deficient cells (Popuri *et al.*, 2012).

RTEL1 is a human functional homolog of Srs2 that suppresses HR and disrupts preformed D-loops (Barber *et al.*, 2008). RTEL1 can also promote synthesis-dependent DNA strand annealing to direct non-crossover outcomes during mitotic (or meiotic) repair of DSBs (Youds *et al.*, 2010). Recent studies also indicate that RTEL1 may suppress recombination in telomeres as well (Uringa *et al.*, 2011). Specifically, RTEL1 disassembles telomeric loops and counteracts telomeric G4-DNA to ensure telomere stability and is also required for telomere elongation (Vannier *et al.*, 2012). Interestingly, *rte1-1*, a *C. elegans* homolog of human *RTEL1* is synthetically lethal with *C. elegans recql5*, with 100% lethality at the embryonic stage (Barber *et al.*, 2008). Double mutant germ lines are characterized by massive accumulation of recombination events: accumulation of RAD51 foci and high frequencies of meiotic recombination, leading to cross-over products (Barber *et al.*, 2008). Although it is still unclear why these double germ lines are synthetically lethal, this phenomenon has been attributed to the inability of cells to reverse nonproductive HR intermediates (Barber *et al.*, 2008). Collectively, these results indicate that RECQL5's backup anti-recombination activity becomes more important in the absence of other recombination suppressors.

Although WRN is not thought to suppress HR in the same manner as BLM and RECQL5, it has been proposed that WRN also suppresses illegitimate recombination (Lebel, 2002; Saunders *et al.*, 2008; Yamagata *et al.*, 1998). WS cells exhibit variegated chromosomal translocations (Salk *et al.*, 1981), elevated recombination levels between micro-homology plasmids (Cheng *et al.*, 1990) and an increase in spontaneous formation of RAD51 foci (Pichierri *et al.*, 2001). Although WRN is involved in end-resection during HR (Chen *et al.*, 2003; Saintigny *et al.*, 2002), it also plays an important role in the repair of stalled/collapsed replication forks by suppressing illegitimate recombination (Dhillon *et al.*, 2007; Pirzio *et al.*, 2008; Rodriguez-Lopez *et al.*, 2007). Consistent with this notion, ectopic expression of a HJ resolvase in WS cells enhances viability and proliferation, suggesting that HR intermediates might accumulate during DNA replication in cells lacking WRN (Franchitto *et al.*, 2008; Rodriguez-Lopez *et al.*, 2002). Consistently, a two-fold increase in the frequency of spontaneous HR was reported in mice with helicase domain deficient WRN protein (Lebel, 2002). Furthermore, in agreement with its potential role in telomeric replication (Crabbe *et al.*, 2004), WRN also suppresses telomeric SCEs (Laud *et al.*, 2005). Interestingly, our recent studies demonstrate that RECQL5 could be synthetically lethal with WRN. We observed that RECQL5 plays both co-operative and complementary roles with WRN helicase (Popuri *et al.*, 2012). RECQL5 specifically interacts with WRN (but not BLM) both physically and functionally and the interaction is maximal during S-phase after replicative stress (Popuri *et al.*, 2012).

RECQL5 co-operates with WRN on synthetic stalled replication fork-like structures and specifically stimulates its helicase activity (but not BLM) on DNA forked duplexes. Both RECQL5 and WRN re-localize from the nucleolus into the nucleus after replicative stress and significantly associate with each other during S-phase (Popuri *et al.*, 2012). Moreover RECQL5 is essential for cell survival in the absence of WRN (Popuri *et al.*, 2012). Loss of both RECQL5 and WRN severely compromises DNA replication and elevates RAD51 foci formation, indicating increased illegitimate recombination. Furthermore, the retention of RECQL5 at DNA DSBs was also extended in the absence of both WRN and BLM (Popuri *et al.*, 2012). These observations suggest that RECQL5 provides an essential function in cells lacking BLM and WRN and would further implicate the divergent and complementary roles that these RecQ helicases might play in the cell. We speculate that RECQL5 responds to stress differently in BLM- and WRN-deficient cells. In BLM-deficient cells, the stress likely arises from elevated SCEs and deposition of RAD51 nucleo-protein filaments. In WRN-deficient cells and in normal cells exposed to replication blocking DNA damage, RECQL5 is probably responding to stalled or collapsed replication forks. In the latter case, WRN's catalytic activities may be insufficient to handle the total cellular burden and therefore RECQL5 may provide assistance and stimulation (Popuri *et al.*, 2012). This functional co-operation could also be enhanced by their mutual interactions with other major players such as FEN1, MRN complex or PCNA. For instance, RECQL5 interacts with PCNA and co-localizes with the MRN complex and this association increases upon replicative stress (Kanagaraj *et al.*, 2006). Similarly, previous studies also indicate a physical interaction of WRN with PCNA (Rodriguez-Lopez *et al.*, 2003) and its potential interplay with the MRN complex in cells experiencing replicative stress (Franchitto & Pichierri, 2004). We speculate that although RECQL5 is less processive than WRN, it cooperates with WRN to facilitate proper replication fork progression *in vivo*.

Interestingly, loss of both RECQL5 and WRN results in abnormal or defective separation of the two sister chromatids (Popuri *et al.*, 2012). Efficient sister chromatid pairing or cohesion between the two sister chromatids is established during DNA replication and is fundamental for cell viability (Carson & Christman, 2001; Skibbens, 2009). Upon depletion of RECQL5 in the absence of WRN, we observed severe defects in DNA replication with very few cells in S-phase (Popuri *et al.*, 2012). Further, loss of checkpoint (CHK1) activation would drive cells into mitosis with defective DNA replication and results in loss of cell viability (Castedo *et al.*, 2004; Yin *et al.*, 2008). Our previous studies indicate that WRN plays both upstream and downstream roles to ATM and ATR. In particular, WRN-depleted cells fail to activate pATM, pATR and pCHK1 in response to replicative stress (Cheng *et al.*, 2008; Patro *et al.*, 2011). WRN-depleted cells also progress through S-phase at a faster rate in response to replicative stress, suggesting a defect in the intra S-phase checkpoint (Cheng *et al.*, 2008). Defects in DNA replication can also trigger mitotic arrest and we observe that most of the RECQL5/WRN double mutant cells were arrested at G2/M phase with the onset of apoptosis and polyploidy (Popuri *et al.*, 2012) that are also hallmarks of mitotic cell death (Castedo *et al.*, 2004; Pflumm & Botchan, 2001). Collectively, these observations highlight a novel backup role for RECQL5 in DNA replication and recombination.

Future perspectives

Moving forward, it will be important to determine if the basic subcellular localization of RECQL5 might be important in differentiating its various roles. RECQL5 predominantly localizes to the nucleolus but can re-localize to the nucleus during S-phase after replicative stress (Popuri *et al.*, 2012). On the other hand, we observe that RECQL5's localization to the nucleolus is altered in ATM- and ATR-deficient cells (unpublished results). We speculate that subcellular localization may depend on specific post-translational modification of RECQL5 and/or its interacting partners which would affect its cellular functions and its coordinated involvement in various DNA metabolic processes (Figure 2). It would be interesting to investigate whether post-translational modifications of RECQL5 modulate its role in transcription and/or other cellular metabolic pathways. Our previous studies indicate that depletion of RECQL5 induces a moderate G2/M cell cycle arrest (Ramamoorthy *et al.*, 2012); in this context, it would also be interesting to investigate involvement of RECQL5 with cyclindependent kinases and cell cycle checkpoints.

Kecql5-deficient MEFs exhibit elevated frequencies of spontaneous DNA double-strand breaks and HR events and are prone to gross chromosomal rearrangements (Hu *et al.*, 2007). More importantly, deletion of *Recql5* in mice results in cancer susceptibility. Detailed phenotypic analysis of *Recql5*^{-/-} mice uncovered an age-dependent increase in the incidence of multiple types of sporadic cancers (Hu *et al.*, 2007), but most prominently with gastrointestinal and colonic tumors (Hu *et al.*, 2010). Human RECQL5 haplotypes were also shown associated with colon cancer in a population-based case control study (Heather *et al.*, 2010). RECQL5 confers CPT-tolerance to human colorectal cancer cells and both RECQL5-deficient human colorectal cancer cells and *Recql5*^{-/-} MEFs are hypersensitive to CPT, a prototype of irinotecan, a drug approved by the FDA for treating colon cancer (Hu *et al.*, 2010; Wang *et al.*, 2011). Collectively, these observations indicate that RECQL5 may suppress colorectal cancer and that RECQL5 may also be used as a potential biomarker for the disease (Hu *et al.*, 2010).

Altered telomere length is linked to a number of diseases, including cancer. Single nucleotide polymorphisms in the *RECQL5* gene were inversely associated with telomere length in DNA from immortalized colon cancers cells (Mirabello *et al.*, 2010; Pellatt *et al.*, 2012). Shorter telomere length results in greater likelihood of developing colon cancer (Pellatt *et al.*, 2012). Incorrect HR and defective DSB repair are also associated with short telomeres (Royle *et al.*, 2009; Wu *et al.*, 2003). A missense mutation could result in loss of function of RECQL5 that might impair proper stabilization of stalled/collapsed replication forks at telomeres and would eventually result in telomere shortening (Pellatt *et al.*, 2012). Moreover, it has also been shown that *C. elegans* RECQL5 is synthetically lethal with the telomeric helicase RTEL1 (Barber *et al.*, 2008). Future studies are essential to explore any functional implications of RECQL5 in telomere maintenance, although our previous studies indicate that RECQL5 cannot efficiently disrupt telomeric D-loops (Ghosh *et al.*, 2009).

Loss of RECQL5 impacts both DNA replication and transcription and can lead to excessive recombination events that might eventually result in gross chromosomal rearrangements and higher tumor incidence. With the observation of increased cancer susceptibility in *Recql5*-

deficient mice, it may be possible that defects in RECQL5 could also be associated with a disease in humans, with a rare patient yet to be identified, similar to the first reported patient with ERCC1 deficiency (Jaspers *et al.*, 2007). Thus, we speculate that loss of RECQL5 would thus be characterized by higher levels of transcription that would hinder DNA replication fork progression and eventually elevate recombination events that would be responsible for higher tumor incidence and increased risk of chromosomal abnormalities (Figure 2). This would most likely occur in highly replicative tissues like skin, gut, bone marrow and hematopoietic tissue.

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Figure 1.
Schematic representation of RecQ helicases from various organisms. The common domains and the protein-specific domains are listed at the bottom. RECQL5 has three isoforms, RECQL5α, RECQL5β and RECQL5γ. The length of the proteins indicated at right in terms of number of aminoacids (aa). (see colour version of this figure at www.informahealthcare.com/bmg).

Figure 2.

Human RECQL5 at the intersection of DNA replication, transcription and recombination. RECQL5 inhibits transcription elongation by interaction with the hyperphosphorylated form of RNA Pol II. RECQL5 promotes strand exchange at stalled replication forks. Collision of a replication fork with the transcription machinery might result in stalled/collapsed replication forks. Alternatively, if a fork encounters a blocking lesion (red triangle) in the leading-strand template, the fork might regress and the nascent DNA strands might anneal to form a four-way junction. Replication past the lesion might be facilitated by the strand exchange and uncoupling of leading- and lagging-strand synthesis. The leading strand could be extended using the longer lagging strand as a template. We speculate that the interactions of RECQL5 with WRN and PCNA at these stalled replication forks might then reset the fork and the leading strand would now be able to extend beyond the lesion. RECQL5 acts as an anti-recombination factor by inhibiting RAD51 nucleo-protein filaments. Transcription associated recombination might also occur when transcription machinery collides with a replication fork as an alternative for fork-reset. Loss of RECQL5 might result in collision of transcription and replication machinery that would result in stalled/collapsed replication forks and eventual elevation of recombination events, (see colour version of this figure at www.informahealthcare.com/bmg).

Table 1

Proposed interaction partners of RECQL5 till date.

Protein	Location	Methods	Region	Function	References
FEN1	Nuc	CBD-PD	ND	RECQL5 stimulates flap endonuclease activity	Speina <i>et al.</i> (2010)
TOPO IIa	Nuc	CoIP	ND	RECQL5 stimulates DNA decatenation activity	Ramamoorthy <i>et al.</i> (2012)
TOPO IIIa/b	Nuc	CoIP	ND	ND	Shimamoto <i>et al.</i> (2000)
PCNA	Nuc	GST-PD	542–991	ND	Kanagaraj <i>et al.</i> (2006)
WRN	Nuc	CoIP	ND	RECQL5 stimulates WRN helicase activity	Popuri <i>et al.</i> (2012)
Rad51	Nuc	CoIP, CBD-PD	515–653	RECQL5 disrupts Rad51 filament formation	Schwendener <i>et al.</i> (2010)
Mrell	Nuc	MS, CoIP	ND	RECQL5 inhibits Mrell exonuclease activity	Zheng <i>et al.</i> (2009)
NBS1	Nuc	MS, CoIP	ND	ND	Zheng <i>et al.</i> (2009)
RAD50	Nuc	MS, CoIP	ND	ND	Zheng <i>et al.</i> (2009)
RNAP II	Nuc	MS, CoIP	KIX, SRI	RECQL5 inhibits RNA Pol II transcription	Aygun <i>et al.</i> (2008), Islam <i>et al.</i> (2010)
SWI/SNF comp	Nuc	MS, CoIP	ND	ND	Zhou <i>et al.</i> (2010)
PARP-1	Nuc	CoIP	ND	PARP-1 regulates RECQL5 helicase activity	Tadokoro <i>et al.</i> (2012)

The interaction partners of RECQL5 in various DNA metabolic processes such as DNA replication, transcription and HR. The modes of functional interaction and the interacting domains involved, with the appropriate references are also listed.