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Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging

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Abstract

The maintenance of the stability of genetic material is an essential feature of every living organism. Organisms across all kingdoms have evolved diverse and highly efficient repair mechanisms to protect the genome from deleterious consequences of various genotoxic factors that might tend to destabilize the integrity of the genome in each generation. One such group of proteins that is actively involved in genome surveillance is the RecQ helicase family. These proteins are highly conserved DNA helicases, which have diverse roles in multiple DNA metabolic processes such as DNA replication, recombination and DNA repair. In humans, five RecQ helicases have been identified and three of them namely, WRN, BLM and RecQL4 have been linked to genetic diseases characterized by genome instability, premature aging and cancer predisposition. This helicase family plays important roles in various DNA repair pathways including protecting the genome from illegitimate recombination during chromosome segregation in mitosis and assuring genome stability. This review mainly focuses on various roles of human RecQ helicases in the process of recombination-based DNA repair to maintain genome stability and physiological consequences of their defects in the development of cancer and premature aging.

Keywords

Genome stability; RecQ helicases; Homologous recombination (HR); Double strand break (DSB); Non-homologous end joining (NHEJ)

Introduction

The stability of the genetic material from one generation to another is an important and essential feature for every living organism. The failure to do so efficiently can lead to chromosomal abnormalities, developmental abnormalities, progression of cancer and premature aging. Living organisms encounter different kinds of stresses provided by genotoxic elements from within (products of normal cellular metabolism i.e., ROS) or outside the cell (environmental

factors such as radiation, chemicals, etc.) during the faithful transmission of their genetic material. To counteract these stress responses, organisms have evolved diverse and highly efficient DNA repair pathways. The repair proteins function in a highly coordinated fashion with other DNA metabolic processes during the different stages of the cell cycle to maintain genome integrity in each generation. An overview of the relationship between DNA repair, cellular stress responses, genome stability and aging has been presented in Fig. 1.

One major family of proteins that is actively involved in maintaining genome stability is the RecQ helicases. The RecQ protein family is a highly conserved group of DNA helicases, which have diverse roles in multiple DNA metabolic processes such as DNA replication, recombination and repair (see review by Brosh and Bohr 2007; Hanada and Hickson 2007; Bachrati and Hickson 2008; Sharma et al. 2006). The RecQ protein is evolutionarily conserved from bacteria, yeast and humans to plants. There is only one RecQ homolog in *Escherichia coli* (RecQ) and yeast (namely, Sgs1 and Rqh1 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively), but five RecQ homologs have been identified in both human and mouse. The largest number of RecQ helicases has been reported in plants, with a total of seven RecQ helicases. Therefore, the function of RecQ helicases seems to have adapted to the complexity of genomes present in higher eukaryotes by increasing their number. The domain architecture of RecQ helicase family members from different organisms is shown in Fig. 2. Five human RecQ homologs, called RECQL1, BLM, WRN, RECQL4 and RECQL5, have been identified so far, and three of them have been shown to be associated with autosomal recessive disorders characterized by premature aging, genome instability, and cancer predisposition. Werner syndrome (WS) is associated with defects in WRN protein, Bloom syndrome (BS) is associated with defects in BLM helicase, and Rothmund Thomson syndrome (RTS), RAPADILINO syndrome, Baller Gerold syndrome are all associated with defects in RECQL4.

RecQ helicases play important roles in various DNA repair pathways including double-strand break (DSB) repair and protecting the genome from illegitimate recombination during chromosome segregation in mitosis (Chakraverty and Hickson 1999; Shen and Loeb 2000; van Brabant et al. 2000). In higher eukaryotes, homologous recombination (HR) is essential for segregation of homologous chromosomes during meiosis, generation of genetic diversity and maintenance of telomeres (Hoeijmakers 2001; West 2003; Krogh and Symington 2004). In addition, recombination-related processes also function in the repair of DNA double-strand breaks (DSBs), interstrand cross-links (ICLs), and recovery of stalled or broken replication forks in DNA replication through a series of interrelated pathways (Fig. 3). Two major recombination pathways have been identified in eukaryotes that are distinct with respect to mechanism and DNA homology requirements. The non-homologous DNA end-joining (NHEJ) pathway joins two ends of the DSB via a process that is largely independent of terminal DNA sequence homology. Therefore, NHEJ is error prone and can produce deletions, insertions and translocations (Thompson and Schild 2002). Homologous recombination (HR) corrects strand breaks using homologous sequences primarily from the sister chromatid and, to a lesser extent, from the homologous chromosome (Johnson and Jasin 2000; Liang et al. 1998). Therefore, it is a high fidelity repair mechanism. These repair pathways involve many proteins whose deficiency results in genome instability, cancer predisposition and premature aging (Ouyang et al. 2008; Li and Heyer 2008). The NHEJ plays a dominant role during G1 to early S-phase of the cell cycle, whereas HR is preferentially used in the late S–G2 phase (Takata et al. 1998), because of the presence of a suitable template (i.e., sister chromatid). The possible roles of different RecQ helicases at the multiple steps of major recombination pathways are represented in Fig. 4.

One of the prominent functions of RecQ helicases is to prevent aberrant and potentially recombinogenic DNA structures that arise as intermediates during DNA replication, repair, or

recombination. RecQ helicases in association with other repair proteins might properly resolve such recombinogenic structures to prevent illegitimate and deleterious crossover recombination. Various RecQ-deficient eukaryotic cells display elevated levels of recombination, suggesting their anti-recombination functions (Bugreev et al. 2007; Hu et al. 2007). This review mainly focuses on the roles of various RecQ helicases in the process of DNA recombination to maintain the integrity of the genome during the faithful segregation and transmission from one generation to the next, and the genetic and physiological abnormalities associated with their defects.

Roles of Werner protein in maintaining genomic stability

Werner syndrome (WS) is an autosomal recessive disease characterized by premature aging associated with genome instability and an elevated risk of cancer. One of the earliest features recognized in primary fibroblasts cell derived from WS patients is limited replicative capacity and reduced ability to proliferate. The WS fibroblasts undergo premature entry into senescence compared to normal cells, which could be one of the reasons for premature aging in WRN patients (Epstein et al. 1965). However, not all cell types derived from WS patients undergo premature senescence. Human T lymphocytes represent a well-characterized example of such a cell type. These cells show no significant reduction in growth capacity compared to normal controls (James et al. 2000), but they are hypersensitive to DNA damaging agents, with aberrations consistent with WRN deficiency (Fukuchi et al. 1990). Cytogenetic observations of WS cells show various types of chromosomal aberrations including deletions, translocations, and rearrangements as well as increased spontaneous mutation (Hoehn et al. 1975; Salk et al. 1985; Fukuchi et al. 1989). Various studies have observed that WS cells show selective sensitivity towards many different types of DNA damaging agents such as 4-nitroquinoline-N-oxide (4-NQO, causing replication fork stalling), camptothecin (CPT) (causing replication fork collapse), ICLs, and ionizing radiation (IR) (Okada et al. 1998; Poot et al. 1999, 2002; Ogburn et al. 1997; Pichierrri et al. 2000). Thus, the WS cellular phenotype is dependent on the sensitivities of WS cells to different DNA damaging agents and efficiency of DNA repair mechanisms including HR to counteract these damages.

Roles of Werner in genetic recombination

Cellular studies have shown marked reduction in cell proliferation following mitotic recombination in WS fibroblasts (Prince et al. 2001). These findings suggested a role of WRN in mitotic recombination. Prince et al. (2001), showed spontaneous genome instability of WS cells by measuring the capacity for mitotic recombination of the WS fibroblasts cell lines. This group observed that WS fibroblast cell lines failed to resolve recombinant products. Thus, in the absence of WRN, unresolved or disrupted gene conversion products may lead to gene rearrangement or loss mediated by other processes and result in recombination-initiated mitotic arrest, and cell death (Prince et al. 2001).

Several lines of evidence suggest that WRN is actively involved in the homologous recombination pathway. Saintigny et al. (2002), have postulated that the physiological role of WRN protein in the cell is to resolve RAD51-mediated homologous recombination (HR) products, and its failure to do so in WS leads to WS cellular phenotypes such as defective recombination resolution, mitotic arrest, cell death, or genomic instability (Saintigny et al. 2002). In accordance with this hypothesis, it has also been shown that WRN interacts physically and functionally with the homologous recombination mediator/single strand annealing protein RAD52 which has been found at arrested replication forks. Biochemical studies have shown that RAD52 both inhibits and enhances WRN helicase activity in a DNA structure dependent manner. WRN, in turn, stimulates RAD52-mediated homologous strand annealing between complementary sequences. Thus, the coordinated activities of WRN and RAD52 may be involved in replication fork rescue after DNA damage (Baynton et al. 2003).

Another major recombination pathway is the NHEJ pathway that is error-prone rejoining process of DSBs. A defect in this pathway consequently results in a loss or gain of genetic information. Oshima et al. (2002) found extensive deletions at non homologous joining ends of the linear plasmids with incompatible ends when introduced into WS cells. Thus, WRN might suppress extensive nucleotide loss during NHEJ and prevent aberrant DNA repair potentially by stabilizing the broken DNA ends or by direct competition with other helicases or exonucleases (Oshima et al. 2002). Thus, in the absence of WRN, regulatory processes controlling NHEJ may be disrupted and relatively large and potentially oncogenic deletions would be generated, leading to accelerated decline in the fidelity of DSB repair. In a very recent study, it has been shown that WRN physically and functionally interacts with the major NHEJ factor XRCC4-DNA ligase IV complex (X4L4) which stimulates WRN exonuclease activity but not WRN helicase activity. Further, X4L4 is able to ligate a substrate processed by WRN exonuclease, suggesting the functional importance of this interaction (Kusumoto et al. 2008).

Roles of Werner in repair of interstrand cross-links

DNA interstrand cross-links (ICLs) covalently bind the complementary strands of the double helix, thus blocking DNA replication and transcription. ICLs are some of the most cytotoxic and genotoxic DNA lesions known (Akkari et al. 2000). ICLs cause replication forks to stall, eventually leading to generation of one-sided DSBs near the ICL site. In eukaryotes, ICL repair is poorly understood, but is thought to involve a combination of nucleotide excision repair (NER), translesion synthesis (TLS) and/or recombination (De Silva et al. 2000; Zheng et al. 2003). Since cells lacking WRN are hypersensitive to DNA interstrand cross-links (ICLs), WRN is likely involved in the repair of ICLs to restore normal replication forks in the cell (Pichierri and Rosselli 2004).

It has been shown that WRN relocates to the sites of arrested replication induced by ICLs, where it physically and functionally interacts with RAD52 (Baynton et al. 2003). Molecular studies by Cheng et al. showed that WRN cooperates physically and functionally with BRCA1 in cellular response to ICLs. WRN helicase activity, but not its exonuclease activity is required to process the DNA ICLs. The BRCA1/BARD1 complex also associates with WRN and stimulates WRN helicase activity on forked and Holliday junction substrates (Cheng et al. 2006). WRN also cooperates with MRN complex both in vivo and in vitro via its association with Nbs1 (Cheng et al. 2004). Further, Otterlei et al. showed that WRN participates in a multiprotein complex containing RAD51, RAD54, RAD54B and ATR in cells where replication has been arrested by ICLs. These findings suggest that WRN plays a role in the recombination step of ICL repair (Otterlei et al. 2006).

One of the steps in HR is the formation of Holliday junctions (HJs) as a recombination intermediate. Although WRN is able to unwind Holliday junctions, it is unclear whether HJs accumulate in WS cells. Recently, Rodriguez-Lopez et al. (2007) created isogenic WS cell lines expressing a nuclear targeted bacterial HJ endonuclease, RusA and showed that Holliday junction resolution by RusA restores DNA replication capacity in primary WS fibroblasts and enhances their proliferation. Furthermore, RusA expression rescued the hypersensitivity of the WS fibroblasts cell to camptothecin and 4-NQO inducing the formation of a double-strand break and fork collapse (Rodriguez-Lopez et al. 2007). This finding suggests that HJs may persist in the absence of WRN (i.e., in WS cells), leading to illegitimate recombination. Thus, WRN is important in vivo in preventing accumulation of HJs. WRN also promotes the ATP-dependent translocation of Holliday junctions which are consistent with the model in which WRN prevents aberrant recombination events at sites of stalled replication forks by dissociating recombination intermediates (Constantinou et al. 2000). The role of WRN in resolving steps of recombination is supported by the observation that WRN contains an enzymatic property which unwinds Holliday junction structures. In vitro studies have demonstrated that the WRN

helicase activity is able to unwind HJs through a branch migration-like activity (Constantinou et al. 2000; Shen and Loeb 2001; Bachrati and Hickson 2003; Khakhar et al. 2003; Lee et al. 2005).

Roles of Werner in stalled replication forks

WRN also plays an important role in the response to replication fork arrest and its recovery during the S-phase of the cell cycle. Cultured WS cells show poor S-phase progression with much lower levels of DNA synthesis activity and an apparent G1 DNA content (Rodriguez-Lopez et al. 2002). These phenotypes account for the loss of proliferative capacity of WS cells and appear to be responsible for early onset of cellular senescence. It has been further observed that there is significant asymmetry in bidirectional replication fork in the absence of functional WRN protein which suggests that WRN might act to prevent collapse of replication forks or to resolve DNA junctions at stalled replication fork in normal cells (Rodriguez-Lopez et al. 2002; Sidorova et al. 2008). Thus, the WRN protein is important in the elongation stage of DNA replication. Further, as mentioned earlier WRN deficient cells are hypersensitive to clastogens (induces replication fork blockage), DNA interstrand cross-linking agents, camptothecin, and hydroxyurea (HU) (Okada et al. 1998; Poot et al. 2001; Pichierri et al. 2001; Bohr et al. 2001). These findings lead us to propose that the WRN helicase/exonuclease normally acts in the sites of stalled or collapsed replication forks.

Although the trigger(s) recruiting WRN to stalled replication sites are largely unknown, available evidence suggest that WRN involvement in response to replication stress is an ATM/ATR-dependent (Pichierri et al. 2003). In addition, several lines of evidence support the view that WRN might play an upstream role in response to DSBs at replication forks. WRN is required for activation of ATM as well as phosphorylation of downstream ATM substrates in cells with collapsed replication forks (Cheng et al. 2008). WRN also associates and colocalizes with the MRN complex following DNA replication arrest (Franchitto and Pichierri 2002, 2004).

Replication fork stalling or collapse is dependent on whether the fork-blocking lesion is on the leading strand or lagging strand. If the polymerase is kept in the coupled replication complex and skips ahead to the next primer to synthesis a new Okazaki fragment, leaving behind a single strand gap containing the lesion, HR is the preferred gap repair pathway. Another pathway to repair the gap is the generation of Displacement loop (D-loop) structures (recombination intermediates) by invasion of the blocked nascent lagging strand into its sister chromatid and extension of the invading strand by DNA polymerase. The extended D-loop structures are excellent substrates for WRN (and BLM helicase) and the dissociated D-loops would be used as a substrate for replication restart. A recent biochemical study suggests that WRN protein catalyses fork regression and HJ formation on a model replication fork in an ATP dependent manner (Machwe et al. 2007). Further, WRN exonuclease activity enhances regression of forks with smaller gaps on the leading arm. This finding suggests that WRN might regress replication forks in vivo, proposing a role for WRN in the recovery of replication arrest (Machwe et al. 2007).

Roles of Werner and other RecQ helicases in telomeric maintenance and preservation

Increasing evidence suggests that telomeric dysfunction is likely to be an important factor for premature senescence and decreased replicative capacity observed in WS cells. Consistent with roles of other RecQ helicases at the telomeres, human BS cells also show telomere defects (Lillard-Wetherell et al. 2004). Du et al. (2004) found that *Wrn* and *Blm* mutations, introduced in a telomerase null mice, accelerates the onset of the pathological phenotype, which includes increased telomeric loss and chromosomal end fusion, which is normally observed very late

in telomerase null mice (Du et al. 2004). These findings suggest important roles played by RecQ helicases in telomere maintenance.

It has been shown that most human somatic cells do not possess sufficient telomerase to maintain telomere length intact through successive generations and in the absence of telomerase, telomeres progressively become shortened in each generation and eventually become dysfunctional, leading to genomic instability, growth arrest, and apoptosis (Harley et al. 1990; Blasco 2005; Opresko 2008). The primary role of telomeres in the cell is to protect the ends of linear chromosomes and prevent them from being recognized as double strand breaks (DSBs) by the cellular damage response machinery. The telomere protects the ends of the linear chromosome by forming a “telosome complex” consisting of six member core proteins and telomeric DNA (de Lange 2005). In humans, telomeres contain 2–10 kb of TTAGGG tandem repeats and end in a 3′ single strand G-rich tail that serves as the substrate for telomerase. The telomeric proteins remodel the telomere ends into a structure that sequesters the 3′ tail and protects them from degradation by nucleases, or prevents them from forming aberrant structures. Existing evidence supports a model whereby telomeric proteins modulate t-loop formation (lasso structure) in which the 3′ tail invades the telomeric duplex and forms a recombination-like D-loop (Griffith et al. 1999, see also Fig. 3iv). Loss of telomere structure and function induces a DNA damage response that involves several proteins that normally respond to the process of DSBs (de Lange 2005). Moreover, WRN and BLM proteins interact physically and functionally with at least three critical members of telosome core complex, namely TRF2 and TRF1 which bind duplex telomeric DNA, and POT1 which binds single stranded TTAGGG repeats and protects the 3′ end of the telomere (Opresko et al. 2002; Stavropoulos et al. 2002; Machwe et al. 2004; Lillard-Wetherell et al. 2004).

RecQ helicases have been implicated in the telomere-based DNA damage response that is induced by mimics of telomeric ssDNA tails (Eller et al. 2006). Studies in yeast have shown the involvement of RecQ helicase protein in homologous recombination pathways at the telomeres. In budding yeast, RecQ helicase (Sgs1) functions in an alternative pathway for lengthening of telomeres (ALT) that occur via recombination in type II survivors of telomerase-negative mutants (Johnson et al. 2001; Huang et al. 2001). Evidence for an ALT-like pathway has also been detected in telomerase-negative mammalian cells from tumors or upon SV40 transformation (Yeager et al. 1999). WRN protein colocalize with telomeric DNA in human cell lines that maintain telomeres by ALT (Johnson et al. 2001; Opresko et al. 2004). The precise mechanism of the ALT pathway in human cells is poorly understood, but several models have proposed the involvement of intra- and intertelomeric D-loop formation. For example, the 3′ telomeric tail invades telomeric duplex DNA in the same telomere (t-loop/D-loop), the sister chromatid, or in a separate chromosome (Neumann and Reddel 2002). A DNA polymerase initiates synthesis at the 3′-OH of the invading strand and increases the length of the telomere, followed by dissociation of the recombination intermediate. It has been postulated that the initiation of telomeric recombination may signal WRN recruitment to either suppress recombination or to dissociate intermediates to ensure proper separation of the recombining telomeric strand. In yeast, RecQ helicase Sgs1 acts in the resolution of recombination intermediates in telomerase deficient yeast strains near senescence, when critically shortened telomeres undergo recombination in an effort to restore telomere length (Lee et al. 2007). Failure to resolve these recombinant structures results in rapid senescence. Further data support the suggestion that Sgs1 is involved in the resolution of telomeric recombination intermediates rather than preventing its formation at the initiation stage (Lee et al. 2007).

An elevated level of sister chromatid exchange at the telomere (T-SCEs) has been observed in WS, indicating hyperrecombination at the telomere in the absence of WRN. Cells from late generation (G5) *mTerc*^{-/-} *Wrn*^{-/-} mice with shortened telomeres show elevated T-SCEs compared to cells from G5 *mTerc*^{-/-} *Wrn*[±] heterozygotes, which could be suppressed with

WRN helicase activity (Laud et al. 2005). This increased propensity towards telomeric recombination correlates with an increase in emergence of immortalized clones from G5 *mTerc*^{-/-} *Wrn*^{-/-} cells which necessarily maintains telomeres by ALT, since telomerase is absent (Laud et al. 2005). These findings suggest that WRN normally suppresses sister chromatid exchanges between telomeres in the shortened or dysfunctional telomeres. Indeed, late passage embryonic stem cells from *mTerc*^{-/-} mice display an increased propensity towards T-SCEs as the telomeres become critically short (Wang et al. 2005). Thus, a requirement for WRN and regulation of recombination at the telomeres to suppress ALT may become more crucial as the telomeres shorten.

The proteins of the NHEJ pathway of recombination, i.e., the Ku70/80 heterodimer, also localize to telomeres and function in telomere maintenance. Ku suppresses recombination at telomeres that are made dysfunctional through the loss of telomeric protein TRF2. Deletion of TRF2 in Ku70^{-/-} mouse cells results in an elevated levels of T-SECs (Celli et al. 2006). WRN physically interacts with both POT1 and the Ku heterodimer, which stimulate WRN helicase and exonuclease activities, respectively (Cooper et al. 2000; Opresko et al. 2005). Very recently, it has been shown that POT1 promotes the apparent processivity of WRN helicase by maintaining partially unwound DNA strands in a melted state, rather than preventing WRN dissociation from the substrate (Sowd et al. 2008). Thus, WRN may function in pathways with Ku or POT1 to suppress recombination at telomeric ends.

Physiological consequences of WRN loss and its relation to aging

Taken together, the above findings support a multidimensional role of WRN in protecting the genome from aberrations and instability. The loss of WRN in cells leads to proliferative defects, limited replicative capacity, and premature cellular senescence. These phenotypes could be due to global genomic damage which results in the rapid exit from the cell cycle of WRN defective cells compared to normal control cells (Faragher et al. 1993).

Another important aspect of the aging phenotype is telomeric erosion or dysfunction. Telomeres play an important role in the genome stability and various theories have been put forward suggesting that telomeric dysfunction is directly linked to cellular senescence and the aging process. As discussed above, WRN protein, in co-ordination with other telomeric proteins like TRF1 and TRF2, is directly involved in preventing telomeric erosion and genome instability. The loss of WRN in the cell leads to elevated telomeric deletions and dysfunction. Therefore, another reason for premature senescence behaviors of WRN fibroblasts could be the telomeric driven senescence due to WRN deficiency (Cox and Faragher 2007). In accordance with this hypothesis, ectopic expression of telomerase (hTERT) prevents WRN fibroblasts from undergoing premature senescence and the WS cells become immortalized (Wyllie et al. 2000; Choi et al. 2001). However, there are some significant differences in the gene expression patterns between WS and normal hTERT-immortalized cells, indicating that telomerase expression does not prevent the phenotypic drift, or destabilized genotype, resulting from the WRN defect (Choi et al. 2001). However, immortalization of WRN cells by hTERT suggests that telomere effects are the predominant trigger of premature senescence in WRN cells. Contrary to this hypothesis, work of Baird et al. (2004) using single telomere length analysis (STELA) showed that WS dermal fibroblasts display normal rates of telomere erosion, suggesting that accelerated replicative decline seen in WS fibroblasts does not result primarily from accelerated telomere erosion (Baird et al. 2004).

Therefore, it is likely that the senescence observed in WS cells is a consequence of the combined effects of irreversible cell cycle exit due to genome instability and accelerated telomere-driven senescence, and that there is complex interplay between the two phenomena.

Roles of BLM protein in genomic stability

Bloom syndrome (BS) is an autosomal recessive disorder characterized by growth retardation, sunlight sensitivity and predisposition to the development of cancer (German 1995; Luo et al. 2000). Cellular investigations show that BS is associated with inherent genomic instability (Bachrati and Hickson 2003; Hickson 2003). BS cells show an elevated level of several types of chromosomal aberrations, including breaks, quadriradials and translocations. The hallmark feature of BS cells is highly elevated levels of the frequency of sister chromatid exchange (SCEs), exchanges between homologous chromosomes, and loss of heterozygosity, which can be used as a molecular diagnostic for this disease (Chaganti et al. 1974; German 1995). These reciprocal DNA exchanges arise primarily as part of HR events that occur during repair of DNA damage in the S or G₂ phases of the cell cycle.

Roles of BLM in homologous recombination

BLM forms a part of multienzyme complex that appears to play roles both in the disruption of alternative DNA structures such as quadruplexes and in the resolution of DNA intermediates that arises during homologous recombination. Consistent with its roles in HR, BLM physically interacts with HR proteins RAD51 and Rad51D, as well as with several other proteins involved in DNA repair and DNA damage signaling such as Mus81, MLH1, MSH6, RPA and ATM (Wu et al. 2001; Braybrooke et al. 2003; Beamish et al. 2002; Sharma et al. 2006; Pedrazzi et al. 2003).

The repair of DSBs by HR is a multistep process. One of the key steps in the HR pathway is the formation of nucleoprotein filaments by RAD51 binding to ssDNA resected ends of DSBs (Sung et al. 2003; West 2003). These RAD51 nucleoprotein filaments possess the interesting ability to “search” the entire genome for a homologous duplex sequence and then catalyses the ssDNA strand exchange reaction with the identical strand in the homologous duplex through complementary base pairing, resulting in the formation of a displacement loop (D-loop). This structure facilitates repair synthesis using the intact homologous sequence as the template strand and invading ssDNA as a primer for DNA polymerase during DNA repair synthesis.

Recent studies showed two novel pro- and anti-recombination activities of the human BLM helicase at different stages (Bugreev et al. 2007). In the early phase of HR, BLM disrupts the Rad51-ssDNA filament by dislodging human Rad51 protein from ssDNA in an ATPase dependent manner, thus preventing the formation of D-loop. These data are consistent with the established role of BLM in suppression of HR at an early stage (Bugreev et al. 2007). Evidence also suggests that BLM may act downstream of D-loop formation (Wu and Hickson 2006). HR can proceed down several pathways. Two pathways, known as synthesis-dependent strand-annealing (SDSA) and double Holliday junction dissolution (DJD), result exclusively in the formation of non-crossover products. BLM has been implicated in effecting both SDSA and DJD (Adams et al. 2003; Wu and Hickson 2003). In one case, a D-loop may eventually convert to a double Holliday junction, and is then processed by dissolution of the Holliday junction. However, SDSA requires the dissociation of a D-loop allowing complementary 3' ssDNA tails of the broken chromosome to anneal and be ligated following DNA repair gap filling. Bugreev et al. (2007) showed that BLM may promote SDSA by facilitating D-loop mediated DNA repair synthesis.

Studies have also shown that BLM interacts physically and functionally with the type IA topoisomerase Topo III α , and catalyses a novel reaction in the resolution of recombination intermediates involving Double Holliday junctions (DHJs), termed as “Holliday junction dissolution” (Hu et al. 2001; Wu and Hickson 2003). This reaction gives rise exclusively to non-cross-over products, which fits very well with the role of BLM as a suppressor of SCEs. The BLM-Topo III α pair is tightly associated with a third protein called BLAP75. Attenuation

of BLAP75 levels by RNA interference destabilizes both BLM and Topo III α (Yin et al. 2005). Biochemical analyses have revealed specific and direct interactions of BLAP75 with BLM and Topo III α and a strong enhancement of the BLM-Topo III α -mediated DHJ dissolution reaction by this novel protein (Wu et al. 2006; Raynard et al. 2006). Recently, Bussen et al. (2007) demonstrated that BLAP75 in conjunction with Topo III α greatly enhances the HJ unwinding activity of BLM. This functional interaction is highly specific, as the BLAP75-Topo III α pair has no effect on either WRN or *Escherichia coli* RecQ helicase activity, nor can *E. coli* Top3 substitute for Topo III α in the enhancement of the BLM helicase activity (Bussen et al. 2007).

Role of BLM in rescuing stalled replication fork

BLM also plays an important role in the repair of stalled or collapsed replication fork during the S-phase of the cell cycle. BS cells exhibit abnormal replication intermediate formation, delayed Okazaki fragment maturation and hypersensitivity to various inhibitors of replication (Davies et al. 2004; Lonn et al. 1990). In response to hydroxyurea-induced replicative stress, BLM localizes to repair centers at collapsed replication forks, which are dependent on stress-activated kinases ATM and ATR (Davalos et al. 2004).

When the replication fork encounters lesions on the leading strand, it causes the replication fork to stall. One potential role of BLM at the stalled replication fork is to promote the fork regression after which the nascent leading and lagging strands anneal to create a structure known as a “chicken foot” (Ralf et al. 2006). This structure will facilitate a process known as “template switching” in which the nascent lagging strand is used as a template and the leading strand extends further to bypass the lesion which is later processed by HR. However, the mechanism by which BLM catalyses regression of the replication fork requires further investigation.

As a result of HR-mediated restart/repair of a damaged replication fork, sister chromatids become covalently linked by Holliday junctions, which need to be resolved prior to mitosis. BLM is able to both bind and branch migrate synthetic Holliday junctions. It has been shown in *S. cerevisiae* that loss of Sgs1 results in the accumulation of HR-dependent replication intermediates that resemble Holliday junctions (Liberi et al. 2005) suggesting that BLM might function in resolving Holliday junctions in a TopIII α and BLAP75 dependent manner (Karow et al. 2000; Johnson et al. 2000).

Roles of RECQL4 in maintaining genome stability

Defects in the RECQL4 gene are the cause of three rare autosomal recessive diseases, namely Rothmund Thomson syndrome (RTS), RAPADILINO syndrome and Baller–Gerold (BGS) syndrome (Kitao et al. 1999; Siitonen et al. 2003; Van Maldergem et al. 2006). Rothmund Thomson syndrome is an unusual disorder characterized by poikiloderma, growth deficiency, juvenile cataracts, premature aging and predisposition to malignant tumors especially osteosarcomas (Vennos et al. 1992; Stinco et al. 2008). Most of the RTS patients show mutations in the RECQL4 helicase domain, resulting in truncated protein due to premature termination of protein synthesis (Lindor et al. 2000). Cytological investigations of various cell types derived from RTS patients show genomic instability and chromosomal abnormalities such as trisomy, aneuploidy and chromosomal rearrangements (Vennos et al. 1992; Der Kaloustian et al. 1990; Orstavik et al. 1994; Durand et al. 2002; Anbari et al. 2000). The different cell types derived from RECQL4-knockout mice display an overall aneuploidy phenotype and a significant increase in the frequency of premature centromere separation (Mann et al. 2005). These results suggest a role of RECQL4 gene in preventing tumorigenesis and maintenance of genome integrity in humans.

There have been contradictory reports of sensitivity to different genotoxic agents of patient-derived RECQL4-deficient fibroblasts. In two independent studies, RTS cells showed sensitivity to H₂O₂, which creates oxidative damage, and ionizing radiation (Werner et al. 2006; Vennos and James 1995), resulting in irreversible growth arrest, decreased DNA synthesis and concomitant reduction of cells in S-phase compared to normal fibroblasts (Werner et al. 2006). However, in another recent study, primary RTS fibroblasts showed no sensitivity to wide variety of genotoxic agents including ionizing or UV radiation, nitrogen mustard, 4-NQO, 8-MOP, Cis-Pt, MMC, H₂O₂, HU, or UV plus caffeine, suggesting the complexity of various RTS cells towards genotoxic responses (Cabral et al. 2008). In another interesting report, it has been shown that, compared to wild type (wt) fibroblasts, primary fibroblasts carrying two deleterious RECQL4 mutations have increased sensitivity to HU, CPT, and doxorubicin (DOX), which exert their effects primarily during S-phase, suggesting a major role of RECQL4 protein in DNA replication (Jin et al. 2008). Further, RTS cells showed modest sensitivity to other DNA damaging agents including ultraviolet (UV) irradiation, ionizing radiation (IR), and cisplatin (CDDP) (Jin et al. 2008). The RTS cells also showed relative resistance to 4-NQO, unlike WS and BS cells which are hypersensitive to this drug (Jin et al. 2008). Mutant human cells lacking RECQL4 escaped from the S-phase arrest following UV or HU treatment, whereas BLM-defective cells exhibited a normal S-phase arrest following UV irradiation (Park et al. 2006). RECQL4 also formed discrete nuclear foci coincident with the nucleotide excision repair factor XPA, in response to UV irradiation and 4-NQO, suggesting that it could be involved in efficient removal of UV lesions (Fan and Luo 2008). However, the discrepancies among different reports might be due to different experimental approaches that have been employed for these studies. These results indicate functional differences among RecQ helicase family members in their possible involvement in various DNA repair and replication pathways.

The cellular functions of RECQL4 are largely unknown. However, data arising from its sensitivity towards different genotoxic agents are indicative of its involvement in distinct DNA metabolic and repair pathways. RECQL4 has been shown to interact with UBR1 and UBR2, members of a family of E3 ubiquitin ligase of the N-end rule pathway, which is a part of the ubiquitin-proteasome system (Yin et al. 2004).

RECQL4 has been proposed to function in the initiation of DNA replication with its N terminus required for the recruitment of DNA polymerase α (Sangrithi et al. 2005; Matsuno et al. 2006). RECQL4 is also known to interact with Cut5 (a homologue of Dpb11 that is required for loading DNA polymerases onto chromatin (Hashimoto and Takisawa 2003). Further, RECQL4 interacts with poly (ADP-ribose) polymerase1 (PARP-1) which is involved in different pathways of DNA metabolism such as DNA recombination, repair, and transcriptional regulation (Woo et al. 2006). In response to the induction of DSBs by treatment with etoposide, a portion of RecQL4 and Rad51 nuclear foci colocalized, suggesting that RECQL4 plays a role in the repair of DSBs by homologous recombination (Petkovic et al. 2005). However, the mechanistic details of this interaction are unknown.

Roles of RECQL1 in genome stability

RECQL1 is found to be the most abundant of all five human RecQ helicases in resting B cells (Kawabe et al. 2000). Studies in chicken DT40 cells have shown that RECQL1 and RECQ5 have roles in cell viability under BLM-impaired conditions, indicating the redundant function of these helicases (Wang et al. 2003). Recent studies have shown that depletion of RECQL1 makes human cells sensitive to IR or camptothecin, and such cells show a high level of spontaneous γ -H2AX foci and elevated SCE, indicating an accumulation of double strand breaks. Further, its physical interaction with Rad51 suggests that RECQL1 may be involved in the repair of DSB by HR (Sharma and Brosh 2007). Consistent with a role of RECQL1 in

HR, very recently, it has been shown that RECQL1 possesses ATPase-dependent DNA branch migration activity (Bugreev et al. 2008). A specific feature of RECQ1-catalysed branch migration is a strong preference towards the 3' → 5' polarity in both the three and four stranded reactions, which is very unique property of RECQ1. This specific 3' → 5' branch migration activity allows RECQL1 to disrupt recombination intermediates (D-loop) formed by invasion of tailed DNA with the 5'-protruding ends. These D-loops, in contrast to the D-loops formed by invasion of tailed DNA with the 3'-protruding ends, cannot be readily extended by DNA polymerase and therefore may represent unproductive recombination intermediates during DSB repair. Therefore, RECQL1 branch migration may prevent accumulation of these unconventional and potentially toxic intermediates in vivo.

Role of RecQ5 in maintaining genome stability

RecQ5 is one of the members of RecQ helicase family that has not been yet linked to any genetic disease. In both *Drosophila* and humans, RecQ5 exists in different isoforms generated by alternative splicing (Sekelsky et al. 1999). In humans there are three RecQ helicase isomers, RecQ5 α , RecQ5 β and RecQ5 γ . Two of these isomers RecQ5 α and RecQ5 γ , are small and localized in the cytoplasm, while RecQ5 β migrates into the nucleus and exists in the nucleoplasm, like other RecQ helicases (Shimamoto et al. 2000).

Recent studies in mouse models have shown that deletion of RecQ5 results in increased susceptibility to cancer. RecQ5-deleted cells exhibit elevated frequencies of spontaneous double stranded breaks (DSBs) and HR (Hu et al. 2007). Mechanistically, human RecQ5 binds to the Rad51 recombinase and inhibits Rad51 mediated D-loop formation by displacing Rad51 from ssDNA. These results suggest that RecQ5 may minimize gross chromosomal rearrangements (GCRs) and tumorigenesis by suppressing the accumulation of DSBs, and attenuate HR by disrupting the Rad51 pre-synaptic filament (Hu et al. 2007).

The results discussed above suggest that higher organisms have multiple pathways to regulate HR and that members of the RecQ helicase family might have overlapping functions in modulating HR. Studies in chicken B-lymphocyte line DT40 cells showed that both RecQ1^{-/-}/BLM^{-/-} and RecQ5^{-/-}/BLM^{-/-} cells grew much more slowly than BLM^{-/-} cells, indicating that RecQ1 and RecQ5 are involved in cell viability when BLM function is impaired. Moreover, RecQ5^{-/-}/BLM^{-/-} cells also showed a higher frequency of SCE than BLM^{-/-} cells, indicating that RecQ5 suppresses SCE under the BLM function-impaired conditions. These results suggest that RecQ1 and RecQ5 in combination with TOP3 α partially substitute the function of BLM under BLM function-impaired conditions, indicating the existence of functional redundancy between different RecQ helicase members (Otsuki et al. 2008; Wang et al. 2003). However, a contradicting result has been observed in mouse embryonic stem (ES) cells, in which disruption of either *Blm* or the *Recq5* gene resulted in a significant increase in the frequency of sister chromatid exchange (SCE) compared to wild type (wt), whereas deleting both *Blm* and *Recq5* leads to an even higher frequency of SCE (Hu et al. 2005). Further, these authors also show that embryonic fibroblasts derived from *Recq5* knockout mice also exhibit a significantly increased frequency of SCE compared to corresponding wild type controls. These results indicate that BLM and Recq5 have non-redundant functions in suppressing crossovers in mouse ES cells (Hu et al. 2005).

Conclusions and future perspectives

The RecQ helicases are essential parts of cellular machinery engaged in DNA metabolism and genome stability. The presence of five different types of RecQ helicases in human and mouse is an adaptive feature towards complexity in the genome among higher eukaryotes. RecQ helicases are involved in various DNA metabolic pathways and ensure error-free DNA

transactions in each generation. Therefore, defective RecQ helicases lead to diverse chromosomal abnormalities, genomic instability and premature aging. Existing literature suggests that different RecQ helicases have overlapping functions in the DNA metabolic pathways. There is likely to be interplay among RecQ helicases in cellular pathways, with one helicase complementing the function of other in a particular type of stress response; some might have non-redundant functions. In the future it would be very interesting to gain insight into how different RecQ helicases function together to ensure genome stability. Among the RecQ helicases only WRN and BLM have been studied in detail, however, the evidence discussed here suggests that other members are equally important in maintaining genome stability. A major focus in the future would be to characterize the cellular and biological functions of other RecQ helicases in response to various stresses.

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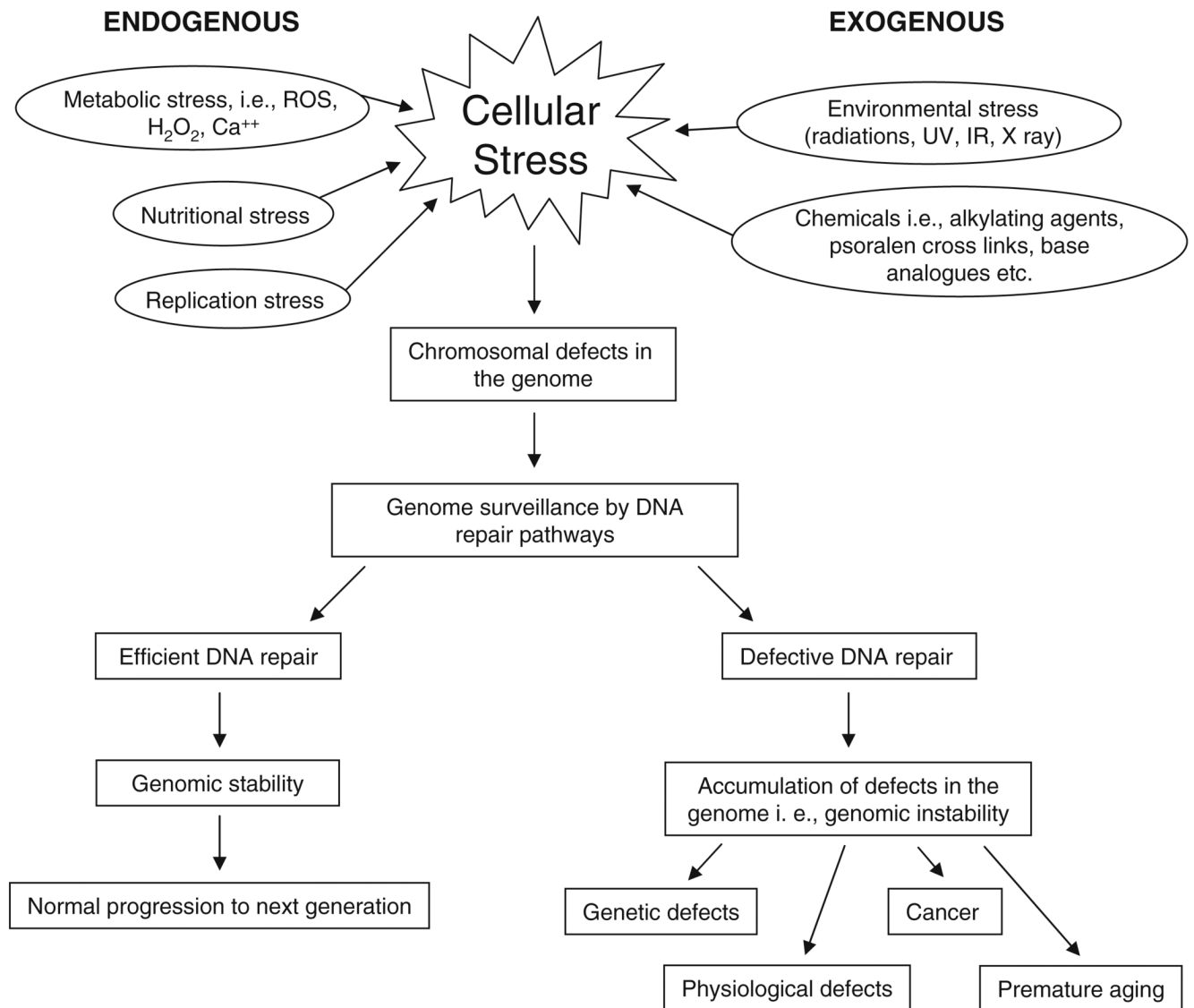


Fig. 1. Overview of relationship between cellular stress responses, DNA repair and genome stability. During the course of their life span, organisms tolerate multiple stresses which might have deleterious effects on their genome. These deleterious effects are encountered by continuous genome surveillance by various DNA repair pathways such as BER, NER, DSB, MMR and mitochondrial DNA repair. Efficient DNA repair machinery in an organism leads to genomic stability, and protects the genome from harmful effects of stress. On the other hand, defective DNA repair machinery would ultimately lead to chromosomal abnormalities which might cause genetic and physiological defects, cellular death, cancer and/or premature aging

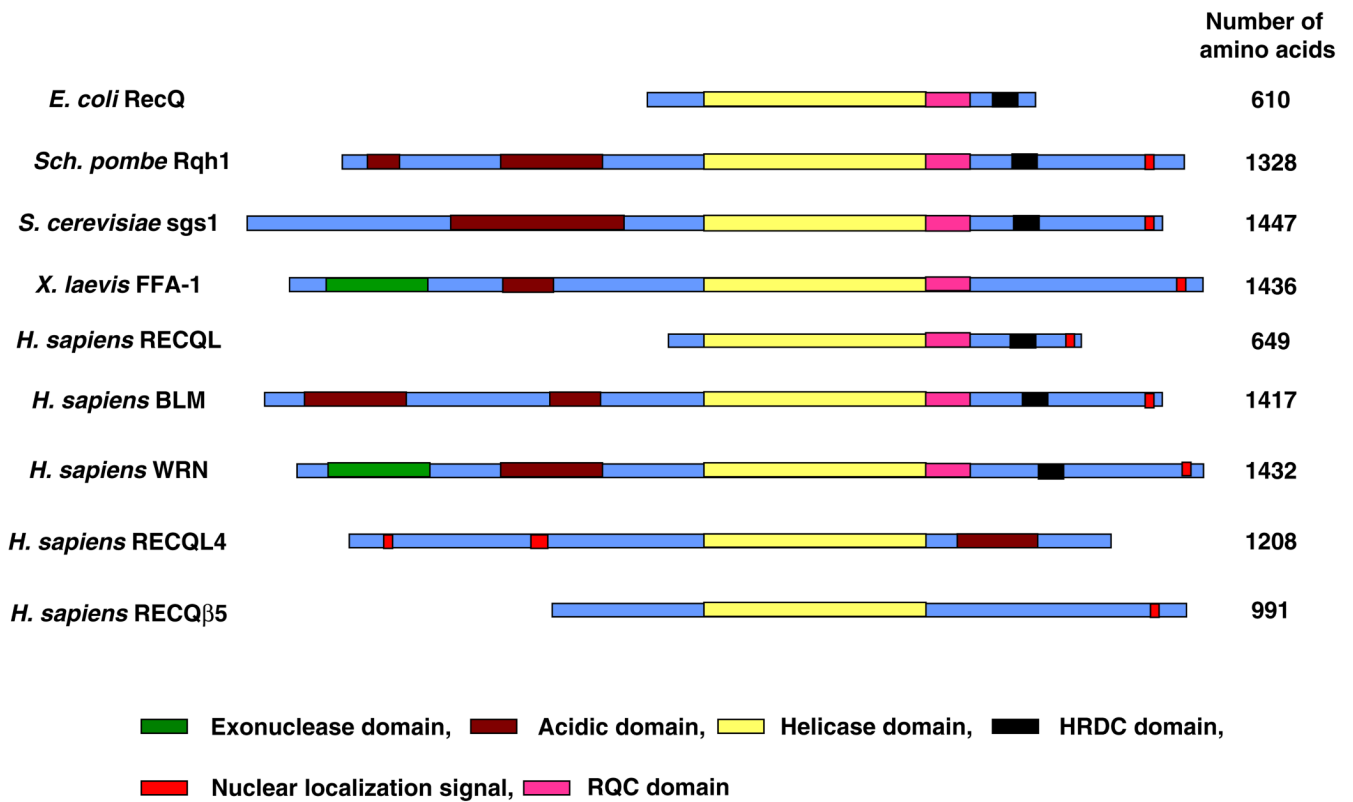


Fig. 2.

Domain architecture of RecQ helicase family. RecQ helicases from different organisms are shown. All the members have a conserved helicase domain in the *central region* of the protein (*yellow*). The nuclear localization signal (*depicted in red*) is present at the C-terminus in most of the family members, except in RECQL4 where it resides at the N-terminus. The WRN protein is unique among human RecQ helicase members in having an exonuclease domain (*green*) at the N-terminus

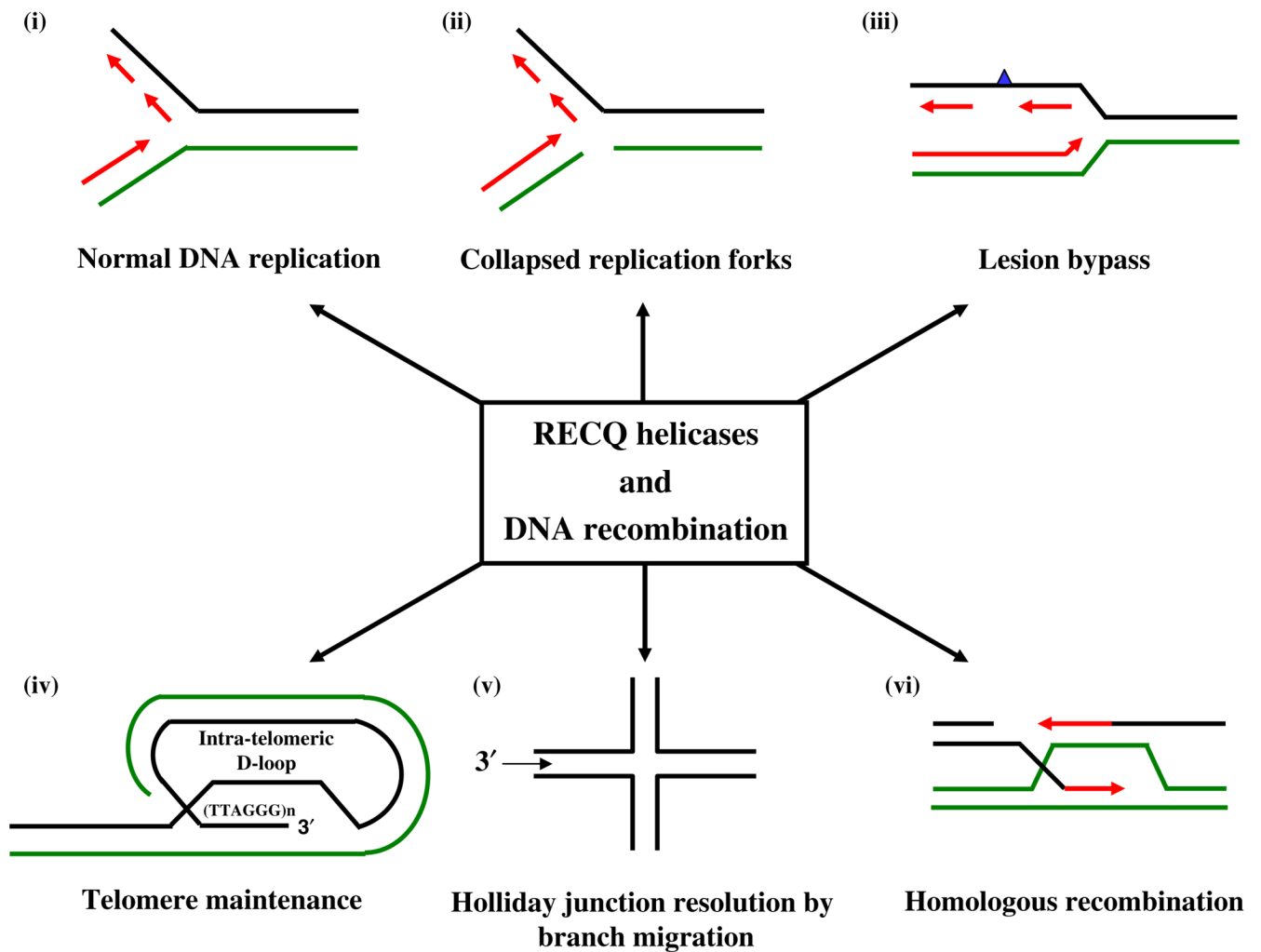


Fig. 3.

RecQ helicases play important roles in various DNA metabolic and repair pathways involving homologous recombination. The RecQ helicases are involved in (i) Resolving aberrant structures encountered at the replication fork during normal DNA replication, (ii) Replication restart at collapsed replication fork sites which arise due to the presence of a nick within the leading strand ahead of replication fork. When the progressing replication fork encounter these nicks (which mimic double strand breaks), it leads to replication arrest. RecQ helicases helps in the replication recovery at the arrested replication fork by promoting fork regression and homologous recombination, (iii) Lesion bypass when the base error is present in the lagging strand. The nick is created due to removal of an incorrectly incorporated nucleotide in the lagging strand which is filled by recombination-mediated gap repair. This is followed by RecQ helicase-mediated resolution of recombinogenic structures (iv) Telomeric maintenance by promoting intra- or inter-strand invasion of 3' tail of the telomere followed by homologous recombination, (v) Resolution of Holliday junctions by branch migration activity, (vi) Homologous recombination and dissolution of Holliday junctions during the process of meiotic segregation and preventing illegitimate recombination during mitosis

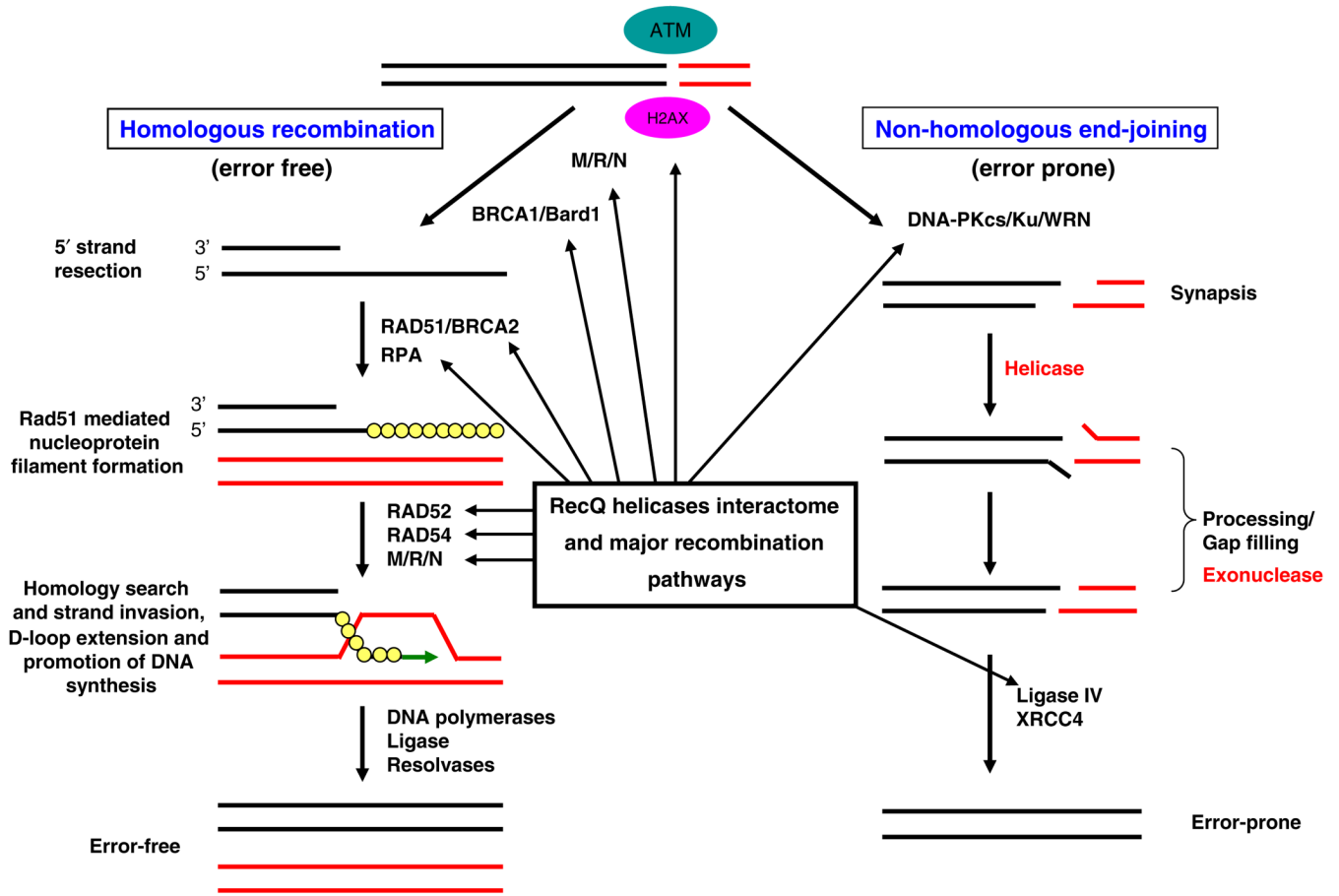


Fig. 4. RecQ helicases are involved in multiple steps of major recombination pathways. The members of the RecQ helicase family interacts with various proteins involved in different steps of the major recombination pathways i.e., error free homologous recombination (HR) pathway and error prone non-homologous end-joining (NHEJ) pathway. (See text for details)