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Distinct functions of human RecQ helicases during DNA replication

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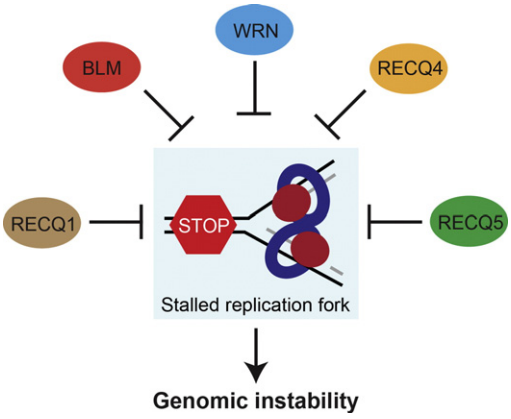
HIGHLIGHTS

- RecQ helicases are essential for maintenance genome stability.
- RecQ helicases have distinct roles in the recovery of stalled replication forks.
- Defects in RecQ helicase function are associated with cancer predisposition.

GRAPHICAL ABSTRACT



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ABSTRACT

DNA replication is the most vulnerable process of DNA metabolism in proliferating cells and therefore it is tightly controlled and coordinated with processes that maintain genomic stability. Human RecQ helicases are among the most important factors involved in the maintenance of replication fork integrity, especially under conditions of replication stress. RecQ helicases promote recovery of replication forks being stalled due to different replication roadblocks of either exogenous or endogenous source. They prevent generation of aberrant replication fork structures and replication fork collapse, and are involved in proper checkpoint signaling. The essential role of human RecQ helicases in the genome maintenance during DNA replication is underlined by association of defects in their function with cancer predisposition.

1. Introduction

Mammalian DNA replication is a well-orchestrated and tightly regulated cellular process that requires enzymatic activities of many factors involved not only in DNA synthesis but also in checkpoint signaling, DNA repair, chromatin remodeling, sister chromatid cohesion and cell cycle control. Defects in any of these activities can cause replication fork slowing or stalling, a condition referred to as replication stress, which leads to genomic instability manifested by birth defects, developmental abnormalities, neurodegeneration, premature aging and cancer predisposition [1–5]. Evidence suggests that members of the RecQ helicase family are among the most important factors that maintain

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genomic stability during DNA replication, especially under conditions of replication stress. The importance of RecQ helicases is underlined by the presence of at least one RecQ family member in all organisms with some exceptions for bacteria and archaea that mostly possess small genomes [6]. Bacteria and yeasts usually contain a single representative of the RecQ family, namely RecQ in *E. coli*, Sgs1p in *S. cerevisiae* and Rqh1p in *S. pombe*. Intriguingly, the number of RecQ family members expressed in particular organism increases with the size of its genome [6]. Non-redundant functions of multiple RecQ homologues in an organism are apparent from their structure. Eukaryotic RecQ helicases share an evolutionary conserved helicase domain flanked by unique N- and C-terminal regions containing interaction sites for other proteins that determine specific functions [7]. In humans, five RecQ homologues have been identified thus far and named RECQ1, BLM, WRN, RECQ4 and RECQ5 (encoded by *RECQL*, *BLM*, *WRN*, *RECQL4* and *RECQL5* genes, respectively). The significance of these RecQ helicases is highlighted by the association of mutations in the genes encoding for BLM, WRN and RECQ4 with severe hereditary disorders named Bloom, Werner and Rothmund-Thompson syndrome, respectively [7]. These rare recessive disorders are characterized by genomic instability and predisposition to cancer (clinical features reviewed in [8–11]). Recently, defects in RECQ1 and RECQ5 have been also connected to cancer development [12–16]. Moreover, the *RECQL* gene has been added on the list of breast cancer susceptibility genes that mostly contains genes involved in the maintenance of the integrity of replication forks like *BRCA1/2* [17,18].

Despite the accumulating evidence for the role of human RecQ helicases in the maintenance of genome stability, the underlying molecular mechanisms remain elusive. Human RecQ helicases have been associated with DNA repair by homologous recombination, base excision repair and non-homologous end joining (reviewed in [7]). However, recent studies have particularly demonstrated the involvement of human RecQ helicases in different aspects of DNA replication. In this article, recent contributions to this topic are reviewed with emphasis on the role of human RecQ helicases in the processing of stalled replication forks.

2. Role of RECQ4 in the initiation of DNA replication

The initial step of DNA replication is the assembly of origin recognition complex (ORC1 to ORC6 subunits) in late mitosis or early G1 phase [19]. Next, CDC6 (cell-division cycle 6) and CDT1 (cdc10-dependent transcript 1) assist to load an inactive double hexamer of minichromosome maintenance proteins 2–7 (MCM helicase) to complete the formation of pre-replication complex required for the establishment of bidirectional replication forks [19]. At the G1/S boundary, S-phase specific DDK (DBF4-dependent kinase) and CDK (CDK2/cyclin A/E) kinases activate MCM helicase, which requires the recruitment of CDC45 and the GINS complex to form the so-called CMG complex (CDC45-MCM-GINS) [19]. Activation of the CMG complex at origins of replication is achieved by recruitment of additional initiation factors including TOBP1, Treslin/TICRR and MCM10, and the loading of DNA polymerase α/ε permit DNA synthesis [19]. Evidence suggests that RECQ4 is among these essential initiation factors [20–22]. The N-terminus of RECQ4 shares a weak but significant homology to the yeast Sld2 that assists in the recruitment of GINS to replication origins during S phase in a manner dependent on CDK activity [20,23–25]. Indeed, the N-terminal region of RECQ4 was shown to be essential for cell viability [20,26,27]. RECQ4 associates with several proteins involved in replication initiation like the MCM complex, MCM10, GINS, CDC45 [28,29]. The absence of RECQ4 was shown to significantly affect the formation of CMG complex [28,29]. Moreover, other replication factors including RPA, PCNA and particularly DNA polymerase α display reduced binding to chromatin in the absence of RECQ4 [21,23]. RECQ4 is recruited to replication origins at G1/S boundary upon ORC and MCM complex assembly, and forms a complex with CTF4 and MCM10 in a process dependent on the CDK and DDK kinase activities [21,28,30]. The efficient binding of RECQ4 to replication origins followed by their firing may be controlled by the interaction between RECQ4 and MCM10 [21,29–31]. However, the interaction between RECQ4 and MCM10 is not required for cell viability [31]. Interestingly, newly characterized Zn-knuckle motif in the N-terminal region of RECQ4 binds nucleic acids with a preference for RNA substrates over DNA, suggesting a connection between RECQ4 and non-coding RNAs that play an important role in the initiation of DNA replication [32].

3. RecQ helicases associate with replisome components

Slow replication fork movement has been observed in cells depleted for BLM, WRN, RECQ1, but not for RECQ4 and RECQ5 [21,33–37]. However, a decreased proliferation of mouse embryonic fibroblasts lacking RECQ4 was observed [23]. Interaction partners of RecQ helicases indicate their participation in processes associated with replication fork progression. RECQ5 and WRN possess the so-called PIP (PCNA-interacting peptide) motif that mediates interaction with PCNA, one of the key replisome components, and both helicases localize to replication factories in unperturbed cells [38,39]. WRN, RECQ5, BLM and RECQ1 interact with FEN1 flap endonuclease that participates in Okazaki fragment maturation, and all stimulate 5′-flap DNA cleavage by FEN1 *in vitro*, in a manner independent of their helicase activity ([40], reviewed in [41]). Moreover, RECQ1 was shown to facilitate efficient binding of FEN1 to telomeres [40]. WRN interacts with DNA polymerase δ and facilitates copying tetraplex and hairpin structures [42]. Moreover, WRN exonuclease is involved in proofreading during DNA synthesis by DNA Pol δ [43]. WRN can also help overcome DNA lesions during replication by interacting with translesion polymerases and stimulating their action [44,45] and by serving as their proofreader [46]. However, recent studies have shown that rather than forming a stable part of replisome, human RecQ helicases remove replication roadblocks and act to promote replication resumption.

4. BLM promotes resolution of aberrant DNA structures at stalled replication forks

Common feature of cells deficient in BLM is increased frequency of sister chromatid exchanges (SCEs) that are thought to arise from aberrant repair of damaged replication forks [47]. To suppress excessive homologous recombination, BLM is recruited to sites of replication fork stalling in a manner dependent on RNF8/RNF168-mediated ubiquitination of the N-terminal region of BLM and subsequent BLM binding to the ubiquitin-interacting motifs of RAP80 [48]. BLM is required for efficient replication fork restart and suppression of dormant origin firing after replication blockage, which is dependent on its helicase activity and phosphorylation (at Thr99) *via* the ATR/Chk1 pathway [35,49]. Cells derived from Bloom syndrome patients accumulate abnormal replication intermediates and display increased levels of single-stranded DNA and RAD51-containing foci, which is pronounced after replication blockage by hydroxyurea (HU) or aphidicolin (Aph) [50,51]. RAD51 is a central homologous recombination factor that plays an important role in the resumption of stalled and collapsed replication forks [52]. BLM and RAD51 act together during fork recovery [35]. Sumoylation of BLM increases the *in vitro* interaction between RAD51 and BLM, and may regulate the recovery of stalled forks by facilitating RAD51 recruitment or stabilization of its binding to stalled forks, and preventing the accumulation of single-stranded DNA coated by RPA. Accordingly, impaired BLM sumoylation (at lysine K317 and K331) increases fork collapse and cell death [53,54].

Four-way DNA structures called Holliday junctions (HJs) can arise upon replication fork stalling by rewinding the parental DNA strands and annealing of the two nascent strands behind the fork to generate regressed arm [55]. This DNA transaction that is dependent on RAD51 may stabilize the stalled fork and facilitate the removal of the roadblock. The regressed arm also serves as a suitable substrate for replication fork recovery by homology-driven invasion of the re-annealed template