Chapter 3

The MCM and RecQ Helicase Families: Ancient Roles in DNA Replication and Genomic Stability Lead to Distinct Roles in Human Disease

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1. Introduction

1.1. Rationale for comparison of MCM and RecQ helicase families

DNA helicases are currently organized into superfamilies based on their sequence structures and 3-D conformations. Within each superfamily, there are members that have further evolved for specialized functions [1]. There is conservation of RecQ proteins from bacteria to humans. Whereas bacteria have one RecQ helicase, humans have evolved at least five different proteins [2]. The RecQ members belong to the helicase Superfamily II, and as such have the characteristic Rec fold [1]. In this chapter, we will focus on RecQ family members WRN, BLM and RECQL4 (RecQ protein-like 4), which is also referred to in the literature as RECQ4. Eukaryotic and archaeal MCMs belong to the helicase Superfamily VI, and have the AAA+ (ATPases associated with diverse cellular activities) fold [1, 3, 4, 5]. Both Rec and AAA+ folds are based on the ancestral ASCE (additional strand conserved E) fold or an alpha-beta-alpha domain necessary for nucleoside triphosphate binding and catalysis [1, 6, 7]. A rationale for comparison of the RecQ and MCM family members relates to the importance of their activities for genomic integrity. The WRN and BLM proteins as well as other members of the RecQ family are characterized by this feature [8]. Both WRN and BLM are involved in DNA repair and a role for WRN in telomere homeostasis in humans is well established [2, 9]. MCM2-7 proteins, along with cofactors, are thought to function as the eukaryotic replicative helicase [10]. MCM8 [11, 12] and MCM9 [13, 14] are more recently discovered and their roles are less well defined. Although data point to a role for MCM8 in DNA replication, that role may be specialized in higher organisms. In human cells, MCM10 is recruited to chromoso-

mal domains before they replicate and studies in yeast suggest a role in DNA replication, but not as a helicase [15-18]. Members of each family are essential for chromosome homeostasis. When replication forks stall, there may be involvement of members of each family. These proteins have interlocking functions since, for example, a stalled replication fork with attendant MCM proteins can lead to a DNA double-strand break (DSB), which requires RecQ proteins for repair [19].

2. RecQ and MCM family structures

Breaks in double-strand DNA can occur during DNA replication, at specific loci (e.g., at telomeres) and during meiosis. RecQ helicases are implicated in DNA repair based on their involvement in such processes as DNA end resection, branch migration, D-loop processing, Holliday Junction (HJ) and double Holliday junction (dHJ) resolution [2]. RecQ proteins function at multiple steps, both early and late, during repair of DSB [19]. RecQ helicases travel in a 3′ to 5′ direction on ssDNA [20]. The RecQ proteins have an ancient lineage based on an ancestral ASCE fold ($\alpha\beta\alpha$ domain) of distant relation to P-loop NTPase folds. RecQ structural domains include a conserved core helicase domain for binding and hydrolysis of nucleoside triphosphate that is equivalent to the Walker A and Walker B boxes seen in MCM proteins [21]. They have a helicase and RNAase D C-terminal (HRDC) domain thought to mediate structure-specific nucleic acid binding, double HJ dissolution and protein-protein interactions. They also have a RecQ C-terminal (RQC) domain thought to mediate interactions with other proteins, structure-specific nucleic acid binding and metal cofactor binding [22]. Acidic regions present in many RecQ proteins aid in protein-protein interactions. There are also nuclear localization signals in some RecQ proteins (e.g., *H. sapiens* WRN and BLM). There are two RecQ members with an exonuclease domain, one of which is *H. sapiens* WRN. Two members have been functionally characterized as having an N-terminal strand exchange domain, one of which is *H. sapiens* BLM [2].

RECQL4 has been reported to have ssDNA binding and DNA strand-annealing activities. In this single study, RECQL4 did not display substrate unwinding or resolution of substrates resembling replication or recombination intermediates [23]. Recognizable HRDC and RQC domains that are important in BLM activity are missing in RECQL4 [22, 24]. As observed, the ssDNA annealing activity would allow RECQL4 to function during synthesis-dependent strand annealing (SDSA) along with another helicase. RECQL4 could help direct pathway choice during HR in DSB repair through aiding ssDNA annealing activity in non-homologous end joining (NHEJ) [19]. Thus, RECQL4 is similar to other RecQ helicases in its core helicase domain, but its function as a helicase is unclear [23, 25, 26].

AAA+ proteins, including MCMs, have a core molecular motor. Like the RecQ proteins, the AAA+ fold is also based on the ancestral ASCE fold. Acquisition of a catalytic glutamate (Fig. 1) to initiate efficient hydrolysis of ATP marked the emergence of the ASCE division from the ancestral P-loop fold [7]. For further discussion of the glutamate “switch” in AAA+ proteins, see reference [27]. Mechanisms of action are diverse, although members are typi-

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cally oligomeric ring assemblies with inter-subunit communication. The central AAA+ motor has been adapted in evolution through structural changes to the core module and through domains added either N- or C-terminal to the AAA+ core. Activities are facilitated by recognition of protein partners functioning in these diverse events. Thus, AAA+ proteins display a variety of macromolecular remodelling events that are energy-driven by nucleotide hydrolysis thought to be occurring throughout what is typically a hexameric complex assembly [28, 29]. The conserved Walker A and Walker B motifs within the central module mediate ATP-binding and hydrolysis [7, 30-32]. MCM proteins have two active site motifs, the P-loop domain and the lid. Motifs in the P-loop include Walker A, Walker B and Sensor 1. The lid domain contains the arginine finger and Sensor 2. A catalytic site is created by a dimer interface that employs a *cis* P-loop from one subunit and a *trans* lid from the adjacent subunit [4, 33, 34]. A similar catalytic site created at the interfaces between adjacent monomers is also characteristic of the RecQ ATPase core [21].

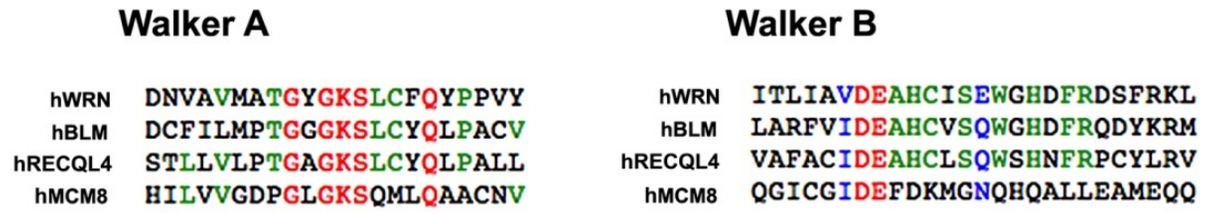

Figure 1. Comparison of conserved ATPase motifs in RecQ proteins WRN, BLM and RECQL4 to the Walker A and Walker B boxes of MCM8. MCM8 was chosen as the MCM for comparison because it has a canonical GKS Walker A [12] and the signature MCM IDEFDKM Walker B ATP-binding domains. In the Walker B motif, note the conserved structural features and the conserved DE motif (containing aspartate, D, and the catalytic glutamate, E).

3. RecQ and MCM helicases: association with disease and aging

3.1. BLM and Bloom syndrome, WRN and Werner syndrome, RECQL4 and Rothmund-Thomson syndrome

As a group, mutations in the RecQ helicases lead to adult segmental progeria with abnormalities in development, predisposition to cancer and acceleration of aging processes. Three of the RecQ family members are associated with rare autosomal recessive diseases [19]. These disorders Werner syndrome (WS) [35], Bloom syndrome (BS) [36] and Rothmund-Thomson syndrome (RTS) [37, 38] are caused by mutations in the genes coding for WRN, BLM and RECQL4, respectively. RTS is a heterogeneous disorder with about 60% of cases resulting from mutations in the *RECQL4* gene [37]. Mutations in *RECQL4* can also lead to two other disease phenotypes [39], but only RTS will be discussed here. The RecQ deficiency diseases are associated with cancer predisposition and several characteristics of aging [8, 20, 26, 40]. In BS cells, there is a 10-fold elevation in frequency of homologous recombination (HR), and reciprocal exchanges occur between homologous chromosomes and sister chromatids [41, 42]. WS cells, on the other hand, display large

chromosome deletions and an increase in illegitimate recombination [43]. A higher frequency of chromosomal aberrations is reported for cells from RTS [44]. These deficiencies thus provide hints as to the cellular activities of these three helicases. Clinical features of these diseases, as referenced above, are as follows.

BS manifests in pleiotropic phenotypes such as growth retardation leading to proportional dwarfism, erythema with light sensitivity, skin lesions with hypo- and hyperpigmentation, immunodeficiency, susceptibility to type II diabetes, male infertility, female sub-fertility, reports of mental retardation, cancer predisposition (all types but at an earlier age of diagnosis than in the normal population).

WS leads to short stature and early onset age-related diseases, including greying hair, alopecia, bilateral cataracts, osteoporosis, arteriosclerosis, atherosclerosis, skin atrophy, hypogonadism, type II diabetes mellitus and susceptibility to tumors, especially those of mesenchymal origin (sarcomas).

RTS manifests as early growth deficiency, congenital bone defects, poikiloderma, cataracts, greying hair, alopecia, hypogonadism, and some increased susceptibility to cancer, especially osteogenic sarcomas.

3.2. MCMs and genomic stability

The MCM proteins 2-7 are necessary for DNA replication in yeast [45, 46], and this basic life function extends in evolution to a single MCM protein in archaea [47-49]. MCM2-7 are also essential for replication in *Xenopus* [50, 51] and have been proposed as a licensing factor for initiation of eukaryotic replication [52, 53]. The MCM proteins 2-8 have an identical Walker B-box motif of IDEFDKM. The MCM2-7 complex is enigmatic in that MCMs 4, 6 and 7 function alone as a heterohexameric helicase [54, 55]. For a discussion of individual MCM subunit arrangements and activities, see the references [33, 34]. MCM2-7 have now been shown to have helicase activity *in vitro* [56], and to be components of a holo-helicase Cdc45/MCM2-7/GINS (CMG) complex [57, 58]. The MCMs require a clamp-loading factor to assemble as a multimeric ring on DNA, and this function is fulfilled in known cases by the protein Cdc6 [59-64] although the regulation of this step in the formation of the CMG complex proceeds through multiple pathways [57, 58]. Various papers have dealt with the function of MCM proteins in DNA replication [10, 46, 65-68], and regarding their processive mechanism of DNA unwinding [27, 56, 58, 69, 70] and only certain lingering, disease-related questions will be considered here. A summary statement can be made regarding known relationships between MCM and RecQ helicases. Members of the MCM protein family are essential for the life-creating process of DNA replication, whereas members of the RecQ family are essential for the life-prolonging maintenance of the genome.

Due to their essential roles, it is not surprising that there are few diseases directly ascribed to defects in MCMs 2-7. This does, however, bring up an unresolved MCM enigma: there are more MCM proteins than are required to form initiation complexes at cellular origins active within a given round of DNA replication [71]. In addition, MCM proteins in human cells remain at peak levels in G2 phase of the cell cycle, after DNA replication is complete [59, 72, 73]

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This high copy number could be an aspect of securing sufficient protein quantities for basic function. A role for MCM proteins in transcription has also been proposed, and this remains to be defined [74]; for further discussion see reference [46]. Redundancy of functions among MCM members is also a factor to be considered. Intriguingly, knockdown of MCM8 [75], not even present in yeast [12], retards S-phase approximately 25% in human cells in culture [75], suggesting a specialized function in higher eukaryotes critical for basic replication. It has been suggested that the excess MCMs license dormant origins, which are used under conditions of stress upon normally functional origins [71]. An overview provides a discussion of recent work connecting dormant origins of replication and tumor suppression based on the role of dormant origins during fork restart after repair of DNA damage [76].

Mutations in MCM family members have been studied in yeast and mouse models, and they confirm an essential role for MCM2-7 in DNA replication [34, 45, 77, 78]. A human MCM4 mutation (destabilizing the MCM2-7 complex) was recently reported concurrently by two groups who studied consanguineous families, and the resulting phenotype was found to be associated with immune deficiency (NK cells), adrenal insufficiency and short stature [79, 80]. Patient fibroblasts showed chromosome fragility [79]. The susceptibility of these patients to cancer is not currently known [80]. An *MCM8* disruption and alternative splice form have been noted in hepatic carcinoma [11] and choriocarcinoma [12], respectively. Although not necessarily a cause of disease, the MCM proteins may be useful tools in diagnoses [81-85]. Elevated levels of MCM proteins 2-7 have been observed in several cancers [81, 84, 85]. In contrast, reduced levels of MCM8 mRNA have been reported in colon carcinoma [12]. Nuclear MCM7 is a good marker for proliferating cells [86], and MCMs 2, 5 or 7 may be an alternative to the Ki-67 marker to distinguish certain hyperproliferative disorders [83].

3.2.1. A structural domain deleted from MCM8 and present in WRN and BLM

A brief discussion of MCM8 is included in this section because it contains a motif that may be structurally similar to one found in BLM and WRN and is linked to neoplasia. Human MCM8 has a splice variant that results in a 16 amino acid (aa) deletion in a location between the Zn finger of MCM8 and its Walker A box [12], Fig. 2. Thus far this deletion has been detected by various different groups only in cases of choriocarcinoma. MCM8 with this same sequence deleted (Fig. 2) has, however, been detected in several higher eukaryotes other than humans, suggesting that the variant does have, or perhaps lacks, a function. That function is as yet unknown, but clues to it may be gleaned from a comparison of the MCM8 deletion with sequences from WRN and BLM. These RecQ proteins have a counterpart 16 aa domain with partial sequence homology and notable structural homology, as denoted in Fig. 2. This sequence in the RecQ proteins is located in a different orientation to that of MCM8 with regard to the helicase Walker A and B boxes (Fig. 2). In each case the first 8 aa of this sequence are highly charged and, in WRN, BLM and MCM8, contain a polar S. The 9th aa in this deletion is a conserved C. The remaining 7 aa contain a preponderance of aromatic and hydrophobic residues. This configuration of charged and aromatic residues is characteristic of known single-stranded nucleic acid binding proteins [87]. Among MCMs, this 16 aa domain is identifiable in the single MCM of *Sulfolobus solfataricus*, in which it has been implicated as a single-stranded DNA-

binding "finger" [88]. Mutations of the positively charged amino acids strongly reduce single-stranded DNA binding of this MCM. In contrast, in BLM the polar S residue is thought to be involved in ATP binding [89]. Because of the aromatic nature of a portion of this domain, binding to one or more DNA nucleotide bases may be involved as a common link between functions of these 16 aa in RECQ and MCM helicases.

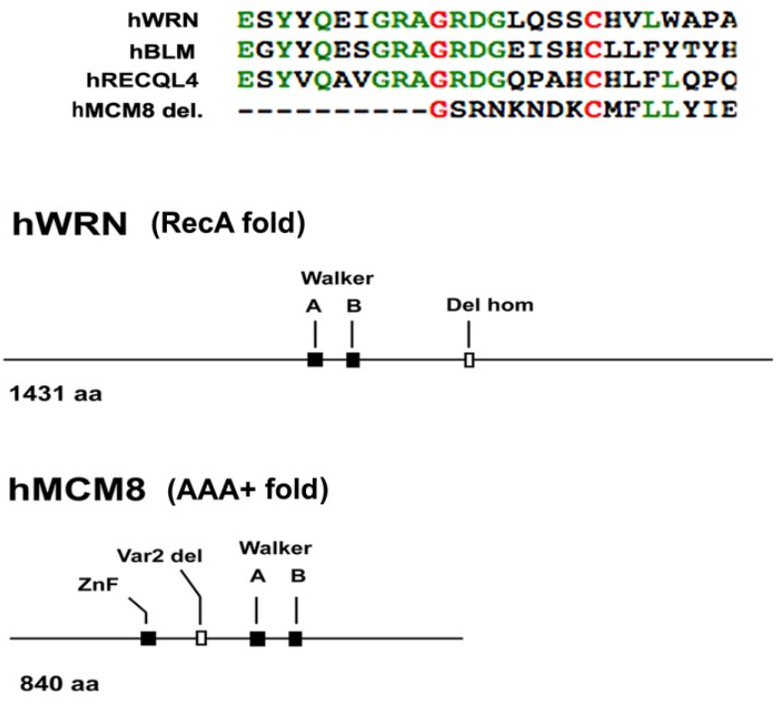

Figure 2. Comparison of placement of selected structural motifs in human RecQ and MCM8 helicases. MCM8 has a splice variant that results in a 16 aa deletion in choriocarcinoma [12]. The Walker A and B boxes and the MCM Zn finger (ZnF) domains are indicated by filled rectangles. The MCM8 variant 2 deletion (Var2 del) is indicated by an open rectangle, as is its partially-homologous counterpart (del hom) in WRN and BLM. This conservatively structured domain consists of an N-terminal highly charged sequence followed by a conserved C and an aromatic-hydrophobic sequence. The open box is located in a different orientation relative to Walker A and B boxes in WRN vs. MCM8. The positions of black and white boxes are approximately to scale.

4. Supportive roles for WRN, BLM helicases and RECQL4 during replication elongation

During normal metabolism, such as in mitochondrial respiration, endogenous reactive oxygen species (ROS) are produced that lead to oxidative DNA modifications. In addition to endogenous mutagens, there are also environmental mutagens that damage the DNA [90]. Furthermore, during replication and transcription, duplex DNA is transiently opened, and there is an opportunity for non-B DNA structures to form in the genomic DNA [40]. RecQ helicases act on recombination intermediates, on preferred substrates including those resembling G-quadruplex DNA [91, 92], D-loops [93], HJ [94] and double HJ [95]. RecQ helicases

may play a regulatory role in both pro- and anti-HR events. They function at the interface of HR with the stressed replication fork and may affect repair pathway choice. There is little evidence for a specific clear cut role, but for a discussion of proposed mechanistic models of RecQ protein function, see the references [19, 20].

4.1. BLM and RECQL4

In BS cells, there are S-phase defects in DNA replication involving abnormal replication intermediates, and replication elongation is slower [96-98]. These cells don't recover well from induced fork stalling and accumulate DSB [99, 100]. In human cells, RECQL4 interacts with the MCM2-7 complex, Cdc45 and GINS. The interaction is facilitated by MCM10 [25]. BLM and RECQL4 are at their highest levels during S phase. At stalled replication forks, BLM physically associates with Rad51 and p53, and BLM and p53 function synergistically in HR [101]. BLM colocalizes in foci with PCNA and with the BASC (BRCA1-associated genome surveillance complex) [102]. BLM is phosphorylated by ATR [103]. When replication forks are stalled by use of hydroxyurea (HU), BLM colocalizes with Chk1 and p53BP1 foci. Chk1 is required for BLM and 53BP1 foci formation. Thus Chk1 may recruit BLM to stalled forks [104]. This implicates RecQ proteins as DNA damage checkpoint mediators in response to stalled forks. *In vitro* studies with use of substrates similar to Okazaki fragments showed BLM stimulation of flap endonuclease [105] (a protein that functions in lagging strand synthesis [106]). BLM functions to prevent the association of homologous sequences in the displaced flap DNA of the Okazaki fragment and the sister chromatid [107-110]. D-loops are formed when a ssDNA tail invades a homologous duplex, and BLM has a preference for dissociating D-loops with a 5′ invaded end suggesting a selection of recombination intermediates that are not extended by polymerase [93, 111, 112]. BLM is able to disrupt the initial Rad51 filament formation step to destabilize recombinase-nucleoprotein filaments. D-loops are susceptible to BLM activity when Rad51 is in an inactive form (ADP-bound) [112, 113]. BLM physically associates with CAF-1 (chromatin assembly factor I) largest subunit, and the colocalization of these two proteins occurs at sites of DNA synthesis. BLM inhibits CAF-1 function in chromatin assembly during DNA repair *in vitro*, and inhibits its mobilization after damage induction *in vivo* [114]. Mammalian WRN and BLM interact [115], and they both interact with RPA [116-118] and p53 [119-121]. Based on coimmunoprecipitation there is limited BLM and WRN interaction, but they may function in the same pathway during HR [20]. BLM helicase activity is stimulated by its binding to the RPA70 kDa subunit [116]. In mouse spermatocytes during meiotic prophase, BLM and RPA are nuclear colocalized [122]. This suggests a potential role for these proteins in resolution of recombination intermediates during meiosis [8]. BLM has a preference for unwinding G-quadruplex structures versus HJ [92]. Both mammalian WRN and BLM bind to G-quadruplex structures, which are roadblocks to polymerases [123, 124].

Aberrant replication intermediates arise in cells lacking WRN and BLM [97, 125]. Such unresolved replication or recombination structures lead to incomplete chromosome segregation. BLM, topoisomerase 3 alpha (Topo3α) and BLAP75/RMI1 (for BLM-associated polypeptide/RecQ-mediated genome instability) or a BLM-Topo3α-BLAP75/RMI1 complex localizes to resulting anaphase bridges [126]. A helicase known as PICH arrives first, followed by the

resolution activity of BLM [127]. As helicases unwind duplex DNA, torsional stress produced in the DNA may require relief through topoisomerase activity, and such activity finally decatenates interlocked DNA molecules [128, 129]. *In vitro* studies show that BLM can partner with Topo3*α* to resolve dHJ and prevent sequence exchange through resolution of this recombination intermediate [95]. The double-junction dissolution reaction requires the HRDC domain of BLM [24]. An additional protein, BLAP75/DMI1 mediates formation of the "dissolvasome" (BLM, Topo3*α* and BLAP75/RMI1) [130-132]. Mammalian BLAP18/RMI2 has also been found to be part of this complex [133].

4.2. WRN

Over 50 distinct *WRN* mutations have been reported, most of which lead to premature termination of translation [19]. Recent missense mutations in the exonuclease domain in one patient compromised protein stability [134]. Most mutations in WS patients occur in the WRN C-terminal domain, which could disrupt the WRN/p53 interaction [20, 134]. Such premature termination could also disrupt the Del hom sequence shown in Fig. 2. No WS mutations have been reported that eliminate only helicase or only exonuclease activity. Both activities are compromised in the development of WS [134]. WS fibroblasts undergo replicative senescence prematurely [135-139]. Telomere defects in WS cells suggest WRN activity in human telomere homeostasis [19]. Telomeres are needed to avoid loss of genetic material. They are important for chromosome end replication and for protection of the ends from enzymatic attack [140]. Human telomeres contain 5 to 20 kb of the repetitive sequence TTAGGG [141]. At the terminal there are 100-200 bp of 3′ ssDNA overhang. This overhang can anneal with telomere DNA to form a stable D-loop leading to a structure referred to as a 't-loop' [142, 143]. Alternatively, this free unannealed end may form G-quadruplex DNA [144]. Human WRN functions in lagging-strand synthesis, and in the replication of telomeric G-rich DNA ends [145]. *C. elegans* WRN-1 can disrupt D-loops [146] and human WRN can prevent aberrant recombination [147]. WRN 3′ to 5′ exonuclease is stimulated by the interacting Ku70/Ku86 complex supporting a role for WRN in DNA repair [148]. Evidence suggests that in the absence of telomerase, WRN and BLM have a role in the ALT (Alternate Lengthening of Telomeres) pathway for telomere maintenance [149, 150]. In biochemical experiments, WRN releases a 3′ invading tail from a telomeric type D-loop by coordinated WRN helicase and exonuclease activities [149].

WRN and BLM catalytic activities are comparable except for 3′ to 5′ exonuclease activity of WRN [151-153], which BLM lacks. On dsDNA and on RNA-DNA hybrids, the WRN exonuclease activity degrades a 3′ recessed end. This activity can remove only one mismatched NT at the end of the recessed 3′ DNA and can initiate exonuclease activity from a gap or nick [154, 155]. The exonuclease activity of WRN can degrade abnormal DNA structures suggesting that WRN helicase and exonuclease activities are involved in resolution of aberrant DNA structures at stalled forks [156]. Human WRN interacts with proteins involved in DNA replication. WRN coimmunoprecipitates with PCNA and topoisomerase 1 [157]. WRN functionally and physically interacts with RPA [117], and it functionally interacts with DNA polymerase delta [152]. WS cells accumulate recombination intermediates that impede cell

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growth [158]. In cells treated with HU, WRN colocalizes with RPA foci and is thought to dissociate recombination intermediates at the stalled forks [94, 147]. WRN stimulates polymerase delta activity in the absence of its processivity factor PCNA. This suggests a role for WRN in recruiting polymerase delta for replication restart at blocked or collapsed forks [152]. RPA can stimulate the processivity of WRN. The stimulation of WRN by RPA is due to protein-protein interactions as opposed to enhanced ATPase activity [117, 118].

5. Unification of BLM, WRN, RECQL4 and MCM2-7 activities in DNA replication and recombination/repair

5.1. BLM: Role in DNA damage response with a complex role in inhibiting or promoting HR

BLM is found mostly in fine granules throughout the nucleoplasm at highest levels during S and G2 phases of the cell cycle. Its focal localization is in PML nuclear bodies (PML-NB). The name PML derives from the promyelocytic leukemia protein, PML [159-162]. This protein forms the structural groundwork of the PML bodies, which store various nuclear proteins [163]. These PML-NB store repair proteins (e.g., Topo3α, MRN and p53) and may be involved in sensing DNA damage [163]. By regulating the availability of repair proteins, response can be directed to DNA damage sites. Trafficking of proteins to the PML-NB is regulated by sumoylation [164]. The sumoylation pathway involves E1, E2 and E3 enzymes, which regulate respectively, SUMO activation, SUMO conjugation and targeting of specific substrates for sumoylation through ligation [165]. BLM contains a motif for SUMO binding that would facilitate its integration into this repair protein storage network [166]. In addition, BLM is SUMO-1 and SUMO-2 modified [167]. When mutants are prepared in which the SUMO-binding sequences are deleted, BLM cannot localize to PML-NB [168]. When mutants are prepared that do not allow BLM localization to PML-NB, there is about a two-fold increase in sister chromatid exchange. These findings indicate that there is a need for BLM-SUMO interaction in order for BLM to localize to PML-NB, and that BLM activity, such as its accumulation at stalled replication forks, may be regulated by this specific localization [168].

At sites of DSB, repair foci form. A central player, H2AX, is phosphorylated when DSBs are induced, and this phosphorylation involves ATM, ATR and DNA-PK. Over one million bp are then marked by phosphorylated H2AX (γH2AX) on each side of the break [112, 168-171]. γH2AX recruits additional repair proteins to the damage site [172]. In studies where normal S-phase cells are treated with DNA damaging agents (HU, UV and cross-linking agents), BLM responds by leaving the PML-NBs to relocate to repair foci and colocalize with the marker γH2AX [101, 173]. BLM interacts physically and functionally with γH2AX as well as with ATM and ATR [103, 173-175]. In damage that is S-phase specific, BLM associates with the complex ATR/CHK1/53BP1, which gathers at repair foci as an early response to the damage. Based on kinetic studies, BLM may facilitate BRCA1 and MRN complex localization at repair foci in S phase [173], which may involve BLM regulation of p53 in these foci [101]. This early function of BLM at repair foci may allow for BML to influence the choice of repair pathways

and facilitate a BLM function in anti-recombination at stalled forks that perhaps involves SUMO regulation [19].

As discussed in the previous section, BLM interacts with the recombinase, Rad51. Rad51 catalyzes the pairing of a ssDNA tail and a homologous stretch of dsDNA to promote strand exchange early in the HR pathway [111]. Following ionizing radiation, Rad51 foci contained BLM [159]. Rad51 functions in HR, and localizes to ssDNA when DSB are induced [176]. BML can displace the recombinase from the ssDNA filament [112], which can be viewed as an anti-recombinagenic function [19]. In Rad51-associated D-loops, BLM can interact with Rad51 and unwind DNA in front of the polymerase [177]. This could favor SDSA leading to pro-recombinagenic function [19, 177]. BLM, however, also functions in resolution of G-quadruplex DNA structure and has higher binding affinity for it compared to HJ. BLM helicase activity is required for resolution of this structure [92]. Whereas the BLM/Rad51 interaction would represent an early event in a DNA damage/repair process, the formation of HJ, on the other hand, is a late event in HR. As discussed in section 5.1, the BLM-Topo3α-BLAP75/RMI1 complex functions to "dissolve" dHJ by convergent fork migration to generate non-cross-over products [178]. This is facilitated by Topo3α relief of superhelicity and by its ability to cleave and rejoin one strand of a DNA duplex. In the absence of BLM, Topo3α activity would involve break and rejoining activities instead of dissolution, which could lead to crossover events and an increase in sister chromatid exchange in BS cells [19]. BLM that is mutated to be unable to interact with Topo3α can only partially rescue the frequency of sister chromatid exchange [168]. Thus, together in a complex, BLM and Topo3α achieve dissolution of a recombinogenic intermediate. BLM has been proposed to regulate ploidy based on a role along with other members of this complex in resolution of anaphase bridges [126]. BLM has been found in complexes with mismatch repair protein MLH1 [179, 180]. It has been found to be present in large complexes containing not only BLM-Topo3a-BLAF75, but also additional factors. These additional factors could include several BLAF factors, proteins from the FA (Fanconi anemia) pathway, RPA, and mismatch repair protein MLH1. [181, 182]. The interactive role of these pathway components remains to be determined.

5.2. WRN: Helicase and exonuclease activities in concert

When WRN biochemical activities were compared using nonhydrolyzable ATPγS to inhibit only WRN helicase activity or aa substitutions to eliminate only the WRN exonuclease activity, the exonuclease activity was shown to function in degradation of the leading strand on replication fork-type substrates and in degradation of the annealed telomere overhangs on substrates resembling D-loop structures. WRN binding proteins were inhibitory [149, 183]. In addition, WRN was found to degrade ssDNA substrates longer than 40 nt with dependence upon the helicase activity [183]. WRN activities were also explored using WS fibroblasts. In WS cells, WRN and the enzyme telomerase are able to reverse the phenotype of excess chromosome fusion. In these cells, the anaphase bridges were missing telomere DNA. Dominant negative telomerase was not able to rescue the phenotype indicating that a stable telomere length was needed for rescue [184]. Telomeres are stabilized by a complex of proteins that bind DNA, known as the shelterin complex [185]. This complex consists of TRF1, TRF2 (double-strand DNA binding proteins), and POT1 (single-strand DNA binding protein) as well as

adaptor proteins. The coordinate action of these proteins in the presence of telomerase is needed to regulate telomere length. Without telomerase, telomeres shorten in length each cell division [185]. In S phase, WRN colocalizes with TRF2 [145, 186]. TRF1 and TRF2 limit WRN exonuclease activity on synthetic telomere D-loops [149]. WRN helicase is stimulated by single-stranded DNA binding proteins, RPA or POT1. These proteins modulate WRN exonuclease degradation of the 3' overhang [149, 187]. The hypothesis is that WRN could unwind D-loops to facilitate leading strand synthesis through telomeric DNA, and there is also the possibility that WRN prevents interchromosomal interactions between telomeres [19].

5.2.1. WRN activities in response to G-quadruplex structures in the lagging strand

At the telomere, the G-rich strand is duplicated by lagging-strand synthesis. It may assume a G-quadruplex structure, which would interrupt the replication fork. Experimentally, when inhibiting WRN by use of overexpression of a dominant-negative WRN, the helicase deficiency leads to loss almost entirely affecting the sister telomere on the lagging strand [145]. WRN interaction with FEN-1 (the flap endonuclease involved in the processing of Okazaki fragments, [188]) could assist in the maturation of these fragments [105, 189]. Data suggest that WRN may function in concert with lagging strand synthesis perhaps in unwinding complex structure at the telomere. WRN interacts physically with DNA polymerase delta to stimulate its activity [190]. WRN can prevent stalling of polymerases delta at telomere sequence *in vitro* [191]. WRN stimulation of polymerase delta happens only in the absence of PCNA, which suggests that WRN has a role at stalled forks rather than in the regulation of processive DNA synthesis [19]. WRN, as opposed to BLM, is specific for G-quadruplex structure in the trinucleotide repeat of Fragile X syndrome [192].

5.2.2. WRN and Ku in suppression of aberrant recombination at telomeres

Proteins involved in the NHEJ path for repair of DSBs that are found at telomeres include Ku heterodimer, DNA-PK, MRN complex and ATM [193]. Through interaction with NBS1, WRN colocalizes with two of these components, Ku and the MRN complex [194]. Under unstable conditions, Ku can suppress sister chromatid exchange at telomeres [195]. Mouse knockout studies show that WRN normally suppresses aberrant recombination at telomeres [196].

5.2.3. WRN activities in base excision repair and interstrand cross link repair by HR

WS cells show increased sensitivity to alkylating agents and to agents that increase ROS. Human fibroblasts in which WRN has been knocked down respond to oxidative stress with an increase in DNA damage [197]. These observations support a role for WRN in repair of such damage [198]. DNA damage resulting from oxidation, alkylation, methylation and deamination is repaired by the base excision repair (BER) pathway. WRN interacts with proteins in this pathway. In addition, WRN helicase activity stimulates and is stimulated by polymerase beta activity [199]. There is evidence that WRN activity in BER is regulated by PARP-1, but the reader is referred to references [200, 201]. BRCA1 and BRCA2 play an important role in DSB repair in the HR subpathway [202, 203]. HR is a pathway for repairing interstrand crosslinks (ICL). Cells deficient in either WRN or BRCA1 are hypersensitive to

induction of ICLs [204, 205]. A physical interaction of WRN with BRCA1 enhances the activities of WRN [206]. In addition, data from WRN and/or BRCA1 knockdown studies indicate that BRCA1 may act cooperatively with WRN in HR during ICL repair [206].

5.3. RECQL4

Data from *Recql4*$^{-/-}$ mice indicate a role for mouse RECQL4 in genomic stability and in the promoting cohesion between sister chromatids [207]. Depleting the *X. laevis* homologue of RTS, xRTS, in *Xenopus* egg extracts led to reduced DNA synthesis and an inhibition of RPA stabilization of ssDNA prior to polymerase loading at unwound origins. The addition of purified human RECQL4 could reverse this effect [208]. A nonhelical region in the N terminus of xRTS could be important in initiation of DNA replication based on its interaction with the Cut5 protein and the importance of xRTS in loading DNA polymerase alpha onto chromatin [209]. The N terminus of xRTS is not homologous to that of RECQL4 in mammals, however, and this role may not be conserved [19]. RECQL4, along with Ctf4 and MCM10, has been shown to be required for stable association of the CMG complex in human cells. In this study, Cut5/TopBP1 was not required for CMG stabilization [210]. In the *Xenopus* replication model, RECQL4 binds to chromatin that has been processed to resemble DSBs with a dependence on RPA and ATM activity [211]. Chromatin immunoprecipitation experiments show that RECQL4 functions on DNA in close association with Ku [212]. In HeLa cells human RECQL4 forms a complex with Rad51 and colocalizes with Rad51 foci formed after treatment with etoposide [212]. PML-NB contain a portion of RECQL4 [212]. It is also found in the nucleolus [213]. When using a T7 phage display screen, RECQL4 was found to interact with PARP-1, and the association influenced the nuclear localization of RECQL4. PARP-1 has a role in RECQL4 movement to the nucleolus from the nucleoplasm. When oxidative stress is induced, as opposed to other types of damage induction, RECQL4 increasingly localizes in the nucleolus. This trafficking is inhibited by inhibition of PARP [213]. RTS cells decrease proliferation and synthesis of DNA when exposed to hydrogen peroxide [214]. Lack of proper response to ROS could lead to premature aging as see in RTS. PARP is also involved in an end-joining pathway of DNA repair [215, 216]. The role of RECQL4 in its interaction with PARP-1 is not known (for further discussion see reference [19]).

5.4. MCM2-7

Replication fork stalling, can lead to DSB and chromosomal rearrangements. An S phase checkpoint is triggered by these events and there is a block to elongation. When this occurs, the proteins Mrc1, Tof1, and Csm3 (M/T/C complex) interact with the MCM2-7 complex to stabilize the replication fork. When the M/T/C complex is missing, the replisome continues, but synthesis stops. This may be partially due to loss of DNA polymerase epsilon from the fork [217-219]. Studies in yeast provide insight into these activities. The M/T/C complex associates with MCM2-7 [218, 220-222] and also with polymerase epsilon [221, 223]. These physical interactions permit communication between polymerase epsilon and the MCM complex [223]. The M/T/C complex may be part of the normal replication fork protein entourage [218, 220, 221, 224]. Mrc1 and Cdc45 coimmunoprecipitate, indicating an interaction of Mrc1 with the The Replication Progression Complex core, which includes Cdc45, the MCMs and GINS [223]. In each cell cycle Mrc loads onto replication origins along with the

polymerases. This occurs after the Replication Progression Complex forms. Mrc migrates with the replication forks. [218, 219, 224-226]. Tof1, like Mrc, also coimmunoprecipitates with Cdc45 [227]. The exact mechanism of action of the M/T/C complex is not known. In a yeast study, the Tof1 homologue could switch regulation between pro- and anti-recombination activities in a site-specific manner [228]. Data indicate that a Mrc1 and MCM6-C terminal interaction senses alkylated DNA damage [221]. The other two subunits Tof1 and Csm3, may function to sense other types of damage. Although the helicase domains of MCM2-7 are conserved, the N and C terminals are divergent. Other negative regulators could differentially bind to these regions to regulate powering of elongation by the MCM2-7 helicase during times of stress [10]. A future question relates to the extent to which leading or lagging strand polymerase arrest is associated with formation of ssDNA, fork regression and formation of abnormal DNA structures [66]. These data indicate a functional connection between the MCM proteins, which act at stalled forks, and the RecQ proteins, which facilitate repair of the resulting damage.

6. Summary

BLM, WRN and RECQL4 act during events that stress the advancing replication fork providing relief through DNA damage repair and through resolution of aberrant replication/recombination intermediates, including those present at the telomere. At checkpoint, the replication proteins at a stalled fork are held stable through communication that occurs due to proteins that bind and signal to both the MCM complex and polymerase(s). This would allow repair proteins such as WRN and BLM helicases and RECQL4 to resolve the stress and thus aid in fork restart. Advancing our knowledge of the RecQ and MCM family activities and the mechanisms and signalling behind these activities will increase our understanding of cancer and aging and perhaps enlighten us regarding how to accommodate these challenges to human health.

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