SURVEY AND SUMMARY

Human premature aging, DNA repair and RecQ helicases

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ABSTRACT

Genomic instability leads to mutations, cellular dysfunction and aberrant phenotypes at the tissue and organism levels. A number of mechanisms have evolved to cope with endogenous or exogenous stress to prevent chromosomal instability and maintain cellular homeostasis. DNA helicases play important roles in the DNA damage response. The RecQ family of DNA helicases is of particular interest since several human RecQ helicases are defective in diseases associated with premature aging and cancer. In this review, we will provide an update on our understanding of the specific roles of human RecQ helicases in the maintenance of genomic stability through their catalytic activities and protein interactions in various pathways of cellular nucleic acid metabolism with an emphasis on DNA replication and repair. We will also discuss the clinical features of the premature aging disorders associated with RecQ helicase deficiencies and how they relate to the molecular defects.

INTRODUCTION

DNA repair pathways

Cellular DNA is continuously being damaged by exogenous and endogenous agents and chemicals, which generate many types of lesions throughout the genome (1). Many of the oxidative DNA modifications that accumulate in nucleic acids over time are thought to be caused by endogenous reactive oxygen species (ROS), which are produced as normal by-products of oxidative phosphorylation in mitochondria as well as other metabolic processes. It has been estimated that between 50 000 and 100 000 oxidative DNA lesions are generated per mammalian cell per day. If DNA damage persists, it can cause errors in DNA replication or transcription leading to point mutations or chromosomal rearrangements, or induce a stress response via various signaling pathways. Cells have multiple DNA repair pathways that provide distinct but overlapping capacity to remove and/or repair many DNA lesions (2) (Figure 1). There are multiple cellular responses to DNA damage and a number of distinct DNA repair processes have been identified. While they represent documented pathways, there is also a great deal of overlap between them and between DNA repair processes and signaling processes elicited by DNA damage.

The four main DNA repair pathways are: base excision repair (BER), which repairs oxidative DNA base modifications such as 8-oxoguanine, alkylation base damage and ssDNA breaks; nucleotide excision repair (NER), which repairs bulky helix-distorting DNA lesions; double strand break repair (DSBR), which repairs dsDNA breaks; and mismatch repair (MMR), which repairs single nucleotide mismatches and small insertion-deletion mispairs. DNA repair subpathways have also been characterized, including the global genome repair (GGR) and transcription coupled repair (TCR) subpathways of NER, long patch (LP-BER) and short patch BER (SP-BER), and homologous recombination (HR) and non-homologous endjoining (NHEJ), which are subpathways of DSBR (Figure 1).

Emerging work has provided compelling evidence for critical roles of DNA helicases in various aspects of DNA repair in addition to their functions in processes associated with DNA replication and recombination. Importantly, RecQ helicases have essential roles in DNA metabolism that directly have an impact on chromosomal stability. Understanding the roles of DNA helicases in the maintenance of genomic stability has become an important challenge since aging, cancer and a number of genetic diseases have been either directly linked or associated with defects in human DNA helicases. Of particular interest has been the role of RecQ helicases, the topic of this review, in chromosomal stability maintenance since our understanding of this family of proteins has advanced significantly over the last decade. Although we

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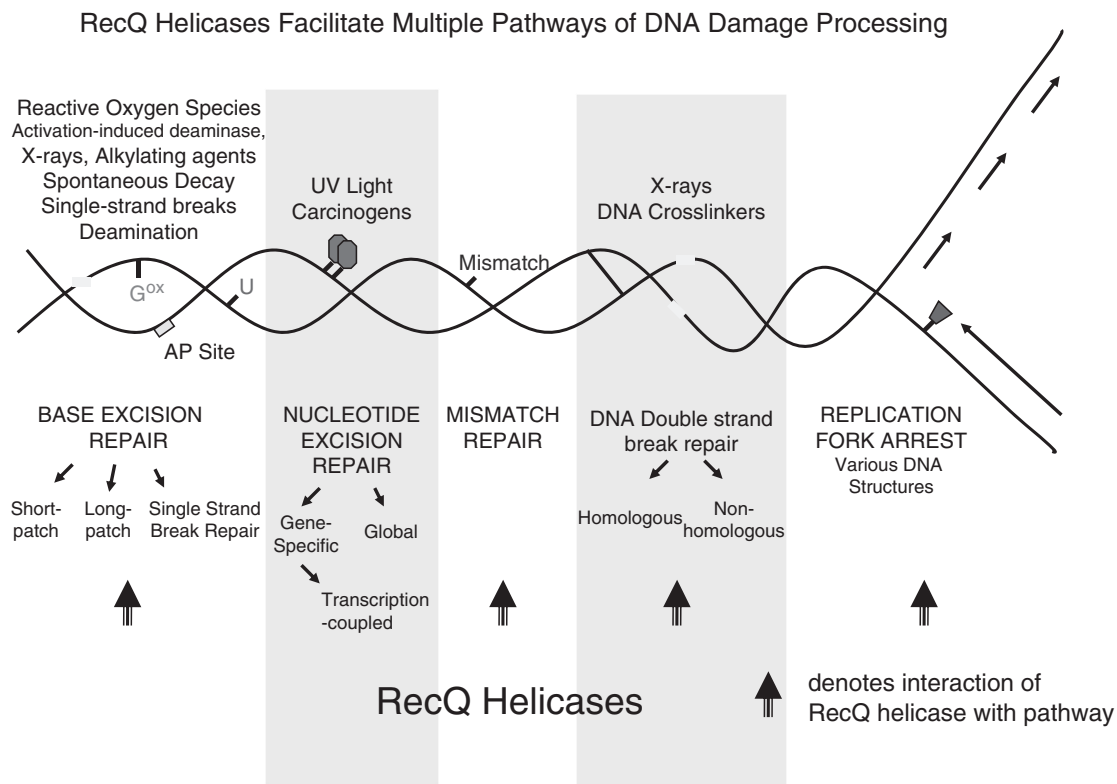RecQ Helicases Facilitate Multiple Pathways of DNA Damage Processing

Figure 1. DNA repair pathways responsible for the correction of endogenous or exogenously induced DNA damage.

have a better appreciation for their roles in DNA repair and genome stability, the precise basis for the molecular and cellular phenotypes associated with mutations or deficiencies in the human RecQ helicases has not been fully realized.

RecQ helicase disorders: chromosomal instability, cancer and aging

Mutations in human RecQ helicases result in rare diseases of aging and cancer characterized by chromosomal instability (Table 1). The most striking clinical feature of Werner syndrome (WS) is the early onset of aging-related diseases (3,4). WS patients have an elevated risk of age-associated diseases such as cardiovascular disease, diabetes mellitus (Type II) and osteoporosis. Unlike normal individuals, WS patients are highly susceptible to early onset of sarcomas and mesenchymal tumors. Bloom syndrome (BS) is associated with an early incidence of most types of cancers, including sarcoma (e.g. osteosarcomas). While the malignancies found in BS reflect what is normally seen in the population, just at an early stage in life, the malignancies found in WS are different from the spectrum in the normal population. WS patients have particularly a high incidence of sarcomas, and this may relate to the hyperoxidation found in WS associated with the BER deficiency (5). The pro-oxidant state in WS (6) and defects in oxidative DNA damage processing (to be discussed) may contribute to the higher levels of oxidation seen in tumors such as sarcomas that occur in this syndrome. Individuals with WS are actually fertile

(albeit hypogonadal) (7), while males with BS are sterile and BS females undergo premature menopause (8).

The incidence of clinical features in WS patients was recently estimated using data from the WS registry (9). Two clinical features, cataracts and voice hoarseness, occur in 100% of WS cases. Interestingly, cataracts are also common in other DNA repair-deficiency diseases, suggesting that lens proteins could be more susceptible to oxidation in patients with these diseases. Gene expression microarray studies using mRNA from WS primary fibroblasts showed an expression pattern more similar to normal fibroblasts from old individuals than to normal fibroblasts from young individuals (10). Despite the fact that WS and BS patients demonstrate early onset of many features of normal aging, not all characteristics of normal aging are accelerated in these patients; thus, WS and BS are known as segmental progerias. There is considerable interest in understanding the pathogenesis of human progeroid syndromes such as WS or Hutchinson-Gilford syndrome (11).

Much work has been done to characterize the cellular phenotypes and genomic instability of WS and BS (3,12–15). BS cells are characterized by higher levels of sister chromatid exchange (SCE) than wild type cells, and both BS and WS cells are characterized by higher than normal levels of chromosomal aberrations and hypersensitivity to various agents that induce DNA damage or interfere with replication, including topoisomerase I inhibitors or DNA interstrand crosslinking agents. BS and WS cells also progress more slowly through S-phase than wild type cells, suggesting that cell

Table 1. RecQ syndromes and mouse models

RecQ Helicase	Human		Mouse	
	Symptom	Cellular phenotype	Symptom	Cellular phenotype
WRN	Premature aging	Genomic instability	None detected	None detected
WRN$^{-/-}$ tert$^{-/-}$	–	–	Premature aging	Telomere dysfunction
BLM	Increased cancer	Elevated SCE	Null, embryonic lethal hypomorphic, increased cancer after 1 year	Elevated SCE, elevated MR, LOH
BLM$^{-/-}$ tert$^{-/-}$	–	–	Tert $^{-/-}$ pathology	Telomere dysfunction
RECQ4	Premature aging	Genomic instability	Growth retardation, skin abnormalities	Sister chromatid, cohesion defect
RECQ5	?	?	None detected	Elevated SCE
RECQ1 (RECQL)	?	?	None detected	Genomic instability, elevated SCE

Note: SCE, sister chromatid exchange; MR, mitotic recombination; LOH, loss of heterozygosity.

cycle or replication checkpoints may be activated in these cells, even in the absence of cellular stress.

Individuals with mutations in RECQ4 are afflicted with Rothmund-Thomson Syndrome (RTS) (16,17). RTS patients display growth deficiency, photosensitivity with poikilodermatous skin changes, early graying and hair loss, juvenile cataracts and osteogenic sarcomas. RECQ4 mutations can also lead to RAPADILINO syndrome (18) or Baller-Gerold syndrome (19). Mutations in the two other human RecQ helicases, RECQ1 and RECQ5, have not yet been genetically linked to a disease; however, studies of mouse model systems (Table 1 and discussed later in the review) suggest that mutations in these RecQ helicases may lead to a hereditary chromosomal instability disorder or predispose individuals to cancer.

Roles of RecQ helicases in DNA repair

Recent studies show that RecQ helicases interact with DNA repair proteins and participate in several DNA repair pathways. This is consistent with the observation that RecQ helicases demonstrate structure-specific DNA binding and have higher affinity for DNA structures that resemble DNA repair intermediates than for simple duplex DNA. A number of recent reviews have summarized the evidence for RecQ helicases in pathways that regulate genetic recombination and DNA repair (3,12,14,20). In this review we will highlight specialized roles of human RecQ helicases, particularly the WRN and BLM helicases, in pathways necessary for genomic stability and the prevention of human diseases associated with premature aging and cancer.

Cellular functions of RecQ helicases mediated by their catalytic activities and protein interactions

The deficiencies in replication and DNA repair characteristics of RecQ mutant cells suggest direct roles of the corresponding RecQ helicases in these processes. A number of biochemical studies from different labs have contributed to our understanding of the structural and catalytic properties of RecQ helicases. The ability of a large number of RecQ helicases to act upon key DNA replicational and repair intermediates (Figures 2A–I)

suggest that these enzymes represent a novel class of proteins to facilitate DNA metabolic functions such as replication restart, recombinational repair and other DNA repair processes such as BER or DSBR. In addition to their catalytic properties, RecQ helicases interact with a number of key protein partners (Table 2) that are implicated in genome stability maintenance through their replication and repair functions. In the following sections, we will highlight what we believe to be some of the most important RecQ functions in terms of the cellular roles of RecQ helicases in DNA metabolism.

The single-stranded DNA binding protein Replication protein A is an important protein partner for human RecQ helicases in the DNA unwinding reaction

A key property of DNA helicases is their ability to unwind long duplexes. Certain DNA helicases (e.g. RecBCD (21) and TraI (22)) can processively unwind DNA tracts of thousands of base pairs, whereas UvrD helicase only unwinds long DNA duplexes in a protein concentration-dependent manner (23). In contrast, the human RecQ helicases, WRN and BLM, are compromised in their ability to unwind duplexes and can only unwind DNA substrates of 100 bp or less in the absence of an auxiliary factor (24). Real-time pre-steady state kinetic studies demonstrate that the WRN helicase unwinds short (19 bp) duplex DNA as a monomer (25); however, the active functional state of unwinding for a number of RecQ helicases remains debatable (14). RecQ helicases such as WRN and BLM may be specifically tailored to act upon short duplex substrates that they encounter during a process associated with DNA repair or replication restart (Figure 2). Alternatively, the RecQ helicase could be associated with another DNA replication/repair protein that serves as an accessory factor, such as Replication protein A (RPA), to facilitate unwinding of longer DNA duplexes that is required during the cellular DNA metabolic processing events. These two possibilities are not mutually exclusive. Given the potential involvement of RecQ helicases in DNA replication, repair and recombination, it will be of interest to determine the role(s) and DNA unwinding mode(s) of these helicases in different sub-complexes.
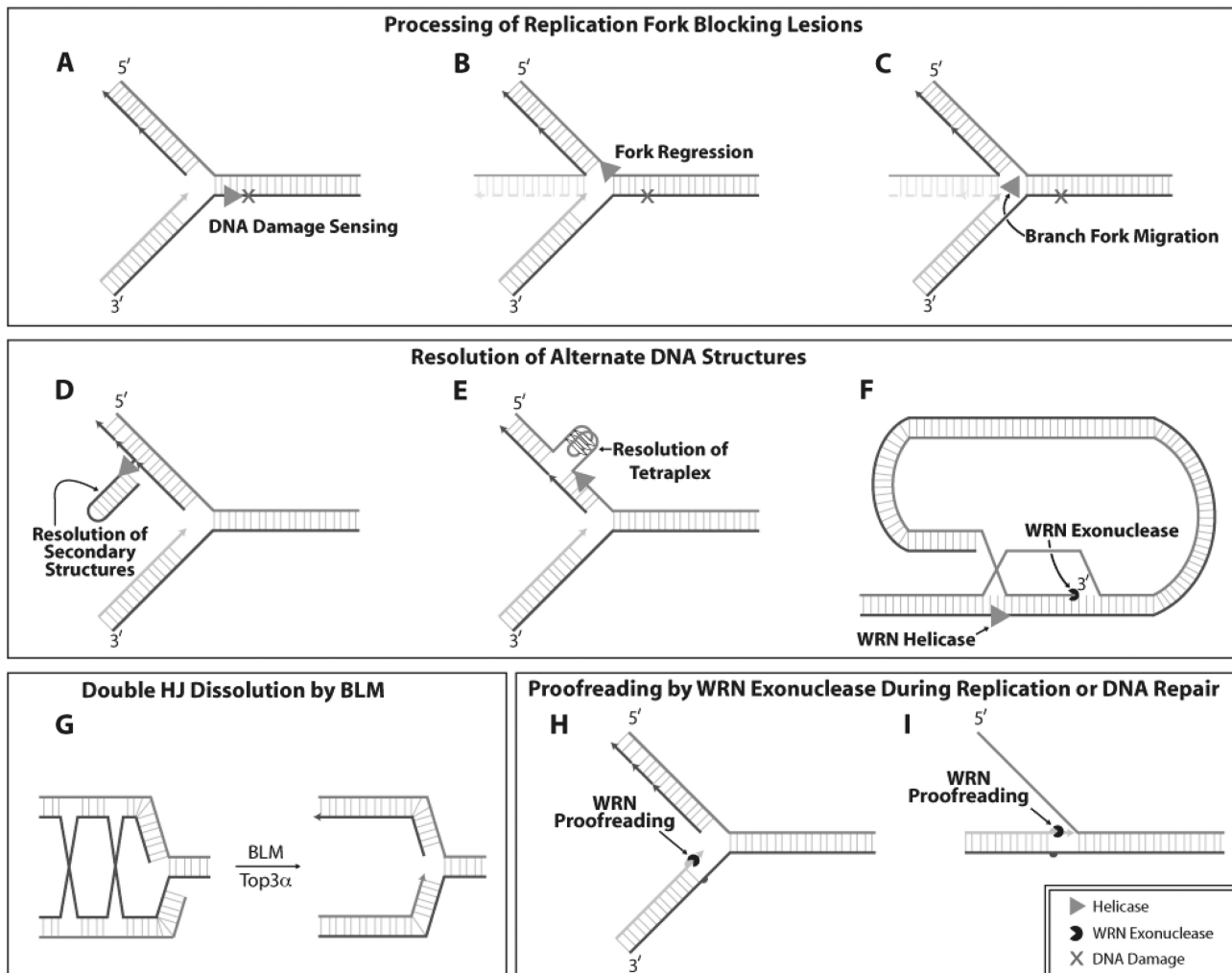
Figure 2. Involvement of RecQ helicases at replication forks and in DNA repair pathways to maintain genomic stability.

Table 2. Highly significant human RecQ protein interactions

RecQ helicase	Interacting Partner	Biochemical and/or biological function	Reference
WRN	RPA	Stimulate WRN helicase, DNA damage response	24,26,27,31
	FEN-1	Stimulate FEN-1 nuclease, processing of replication fork and repair structures	66,67,69–71
	PARP	Inhibit WRN helicase and exonuclease, BER	76–78
	Ku70/80	Stimulate WRN exonuclease, NHEJ	97,98
	TRF2 / POT1	Stimulate WRN helicase, telomere function	126,127,136,137
	Pol δ	Stimulate pol δ DNA synthesis, replication	93,94
	Rad52	Inhibits or stimulates WRN helicase in DNA structure-specific manner, HR	84
	p53	Inhibits WRN catalytic activities, apoptosis	38–41
	MRE11-RAD50-NBS1	Promotes WRN helicase activity, HR repair	85
BLM	RPA	Stimulate BLM helicase, DNA damage response	24, 28
	FEN-1	Stimulate FEN-1 nuclease, processing of replication fork and repair structures	68, 69, 71
	Top3α/BLAP75(RMI1)	Double HJ dissolution, resolve recombination/late stage replication structure	48–52
	CAF-1	Inhibit CAF-1 mediated chromatin assembly during DNA repair	36
RECQ1	RPA	Stimulate RECQ1 helicase	29
	EXO-1	Stimulate EXO-1 nuclease	173
RECQ5β	RPA	Stimulate RECQ5β helicase	30
RECQ4	Rad51	Co-localize upon DSB induction	82

Note: For WRN and BLM, emphasis is placed on protein interactions in which cellular studies implicate biological importance. Additional protein interactions for WRN and BLM have been reported.

Modulation of RecQ-catalyzed DNA unwinding by an auxiliary factor such as RPA may serve to regulate DNA metabolic processes fundamental to replication restart or recombinational repair and/or prevent unnecessary formation of single-stranded DNA that may be deleterious for genomic stability.

The functional interaction and mechanism whereby RPA stimulates the DNA unwinding reactions catalyzed by human RecQ helicases is specific as evidenced by the inability of heterologous, single-stranded DNA binding proteins such as *E. coli* single-stranded DNA binding protein (ESSB) or T4 Gene 32 to enhance unwinding of long (several hundred bp) DNA substrates by human RecQ helicases [for review, see (14)]. The specificity of the RPA interaction was observed for the human RecQ helicases, WRN (26,27), BLM (28), RECQ1 (29) and RECQ5β (30) (Table 2), suggesting a similar mechanism for stimulation. The physical interaction between RPA and human RecQ helicases (WRN and BLM) was shown to play an important role in the mechanism for RPA stimulation of helicase-catalyzed DNA unwinding (24). However, WRN and BLM interact with RPA70 via domains of the helicase that are functionally conserved but do not display extensive sequence homology (24). The interaction of RPA with its partner helicase is likely to be complex. For example, the WRN interacting domain and ssDNA binding domain of RPA overlap with each other (24,31), suggesting that ssDNA and WRN protein-binding domains of RPA70 are functionally intertwined.

In addition to RPA loading a DNA processing protein such as helicase on to ssDNA, RPA interaction with some helicases may enable them to actively place RPA on ssDNA as it emerges from the helicase complex (32). Protein partners of RPA, such as helicases, may trade places on ssDNA by binding to RPA and mediate conformational changes that alter the ssDNA binding properties of RPA. The order for sequential loading of the RecQ helicase and RPA to unwind a DNA intermediate is particularly interesting and challenging to decipher since the precise details for the cellular roles of the human RecQ helicases remain to be defined.

Understanding the biological importance of the interaction of RPA with various RecQ helicases may be highly relevant to deciphering the DNA metabolic defects in RecQ helicase disorders. The coordinate action of RPA and a RecQ helicase such as WRN is likely to play a role in the replicational stress response since the two proteins are known to co-localize at blocked replication forks (33) or sites of DNA damage (34). In addition, RPA may enable the RecQ helicase to unwind past DNA-blocking base lesions (35) (Figure 2A). RecQ helicases may play a role in DNA damage signaling or in chromatin assembly associated with DNA repair (36). RPA is implicated in signaling at stalled replication forks where the accumulated ssDNA is bound by RPA, creating a signal for activation of the ataxia-telangiectasia and rad3-related (ATR)-dependent checkpoint response (37). It will be important to address the role of RecQ protein interactions with DNA repair factors such as RPA that modulates its catalytic (helicase) activity to understand their roles in DNA repair or DNA damage signaling. The ability of the tumor suppressor p53 to modulate RPA-dependent or RPA-independent WRN catalytic activities (38–40) suggests a functional relationship of the proteins in a pathway such as p53-mediated apoptosis (41).

Roles of RecQ helicases to suppress SCEs

From a historical perspective, the roles of RecQ helicases to maintain genomic stability through their interactions with cellular topoisomerases have been well documented. In *E. coli*, the prototype and singular RecQ helicase interacts with Topoisomerase III (Top3) to suppress recombination of newly replicated and catenated daughter DNA molecules before cell division (42,43). Biochemical demonstration of a concerted action of *E. coli* RecQ and Top3 to catalytically link and unlink covalently closed circular DNA molecules suggests a mechanism whereby inappropriately joined DNA molecules can be resolved from one another. In *Saccharomyces cerevisiae*, *sgs1* mutation is a slow growth suppressor of the *top3* mutant (44). Sgs1 and Top3 genetically and physically interact, suggesting a model in which together they suppress cross-over products that form during Holliday Junction (HJ) resolution of recombination intermediates (44,45). In *S. pombe*, the sole RecQ helicase Rqh1 functions with Top3 after DNA replication during G2 to facilitate DNA repair (46). Double strand break (DSB) formation and hyper-recombination at replication fork barriers and defective chromosome segregation in the absence of Rqh1 may be a consequence of the defective RecQ-topoisomerase interaction (47).

The abundant evidence for a direct physical and functional interaction of prokaryotic and lower eukaryotic RecQ helicases with Top3 evoked an interest in the functional importance of this protein interaction in human RecQ mutant cells and disorders. A hallmark for the chromosomal instability of BS is the abundance of quadriradial homologous chromosomes in BS somatic cells that may arise from elevated SCE (3). On average, the number of SCEs from metaphase spreads of BS fibroblasts is increased 7-fold compared to normal fibroblasts. Thus, elevated SCEs are used for the clinical diagnosis of BS. The dramatically increased SCE in BS has sparked an interest in understanding the cellular and molecular mechanism for the peculiar type of chromosomal instability. The conserved interactions of RecQ helicases with topoisomerases provided a framework for investigating BLM protein interactions. In human cells, the BLM helicase forms a protein complex with Top3α in PML nuclear bodies of exponentially growing cells (48–50). An N-terminal region of BLM protein was necessary and sufficient to bind Top3α, but was not sufficient to suppress SCEs *in vivo* (48). *In vitro* studies have demonstrated functional interactions between BLM and Top3α. BLM together with Top3α has the ability to catalyze double HJ dissolution on model DNA substrates in a reaction that requires BLM-mediated ATP hydrolysis and the active-site tyrosine residue of Top3α (51) (Figure 2G). This reaction gave rise

exclusively to non-cross-over products, as predicted from the hemicatenane model, and supports a proposed role of BLM with Top3α as a suppressor of SCEs. Other RecQ helicases (RECQ1, WRN and RECQ5β) fail to substitute for BLM in the *in vitro* double HJ dissolution reaction (52). However, a functional connection between the yeast RecQ helicase SGS1 and hemicatenanes, including in telomere maintenance was recently reported (53–55).

Despite the apparent unique *in vitro* requirement for BLM helicase to perform double HJ dissolution with Top3α, mounting evidence suggests that other RecQ helicases function in non-redundant pathways to suppress cross-overs during mitosis. In chicken DT40 cells, $RECQ5^{-/-}$ $BLM^{-/-}$ cells have a higher frequency of SCE compared with $BLM^{-/-}$ cells (56). Mouse models also suggest roles of other RecQ helicases than BLM in the maintenance of chromosomal stability (Table 1). An independent role of RECQ5 in the suppression of SCEs was demonstrated in embryonic stem (ES) cells or differentiated fibroblasts from *RECQ5*-knockout mice (57). Likewise, elevated SCE in ES cells from RECQL knockout mice was also reported (58), indicating that the RECQ1 helicase is uniquely important for chromosomal stability. It will be a high priority to delineate the functions and pathways of the mammalian RecQ helicases to suppress SCEs since these events lead to mutagenesis and genomic instability that contribute to carcinogenesis.

Proposed roles of strand annealing catalyzed by RecQ helicases in cellular DNA metabolism

In addition to their helicase activity, the human RecQ helicases RECQ5β, RECQ1, WRN, BLM and RECQ4, as well as the *dm*RECQ5β (*dm* is *Drosophila melanogaster*) efficiently catalyze strand annealing of complementary ssDNA molecules [for review, see (14)]. Strand annealing catalyzed by RecQ helicases does not require ATP or free Mg^{2+}. Analyses of strand annealing utilizing ATP-binding/hydrolysis mutants revealed that nucleotide binding inhibits strand annealing catalyzed by either RECQ1 (59) or RECQ5β (30). ATP binding induces a conformational change in RECQ1 protein, which serves as a molecular switch from a strand annealing to a DNA unwinding mode (59). RECQ1 assumes different oligomeric forms to perform its helicase and strand-annealing activities (60). Coordination between the strand pairing and the DNA unwinding activities of WRN or BLM enables these enzymes to catalyze strand exchange between a partial duplex and a complementary third strand. Mapping studies demonstrated that the C-terminal region after the helicase domain of RECQ5β mediates strand annealing (30). The BLM C-terminal region (amino acids 1267–1417) adjacent to the helicase-related C-terminal (HRDC) domain is important for BLM strand annealing and DNA binding (61). These findings suggest that the annealing function is dictated by the poorly conserved C-terminal regions in these RecQ helicases.

An important question remains concerning the biological importance of strand annealing catalyzed by RecQ helicases. The antagonistic catalytic action of strand annealing versus DNA unwinding suggests that these RecQ functions are regulated to perform DNA transactions in specific pathways or steps of a given pathway. Conceivably, RecQ assembly state (as suggested by the RECQ1 oligomeric studies) may influence the enzyme's ability to act upon certain DNA structures. The implicated roles of RecQ helicases for the proper response to replicational stress suggests that this class of enzymes have direct roles in the metabolism of DNA structures associated with a stalled or altogether blocked replication fork (Figure 2A–C). Conceivably, strand annealing by RecQ helicases may be important for replication fork regression (formation of a 'chicken foot' structure) at blocked DNA replication forks. BLM helicase, but not *E. coli* RecQ, was shown to promote regression of a model replication fork *in vitro* (62). Some differences in substrate specificity or directionality of unwinding a synthetic replication fork structure have been observed among the RecQ helicases (63,64), suggesting that these enzymes may have specialized unwinding functions tailored for specific DNA metabolic intermediates. Both WRN and BLM (but not other selected helicases including UvrD, Rep, PriA and *E. coli* RecQ) can coordinate their unwinding and pairing activities to regress a model replication fork substrate.

In the model for fork regression, replication fork uncoupling at a polymerase-blocking lesion in the leading strand template enables the synthesis of an Okazaki fragment past the DNA damage (Figure 2B). This is followed by fork regression in which the nascent leading and lagging strands are annealed and the longer lagging strand becomes a template for leading strand synthesis. The four-stranded HJ structure can be branch-migrated past the lesion to enable replication restart (Figure 2C). Therefore, DNA unwinding and strand-annealing activities catalyzed by RecQ helicases may be important for replication fork regression (formation of a chicken foot structure at blocked DNA replication forks) and restoration of the fork for the restart of replication by a non-recombinogenic mechanism.

In vitro studies suggest that certain RecQ helicases are able to deal with alternative recombination structures (Figure 2D–F). Coordination between the strand-pairing and DNA unwinding activities of WRN or BLM readily achieves strand exchange between a partial duplex and a complementary third strand. A mechanism for the disruption of recombination intermediates of Okazaki fragment maturation by BLM was proposed (65). BLM strand exchange has the ability to efficiently dissociate invading flaps such as those of a D-loop sub-structure provided that the exchange step does not involve annealing of RPA-coated strands. The BLM functions may inhibit illegitimate recombination during Okazaki fragment processing. WRN and BLM both have the ability to stimulate Flap Endonuclease 1 (FEN-1) structure-specific cleavage of fixed (66–69) and equilibrating (70,71) flap structures, the latter being a proposed physiological substrate of FEN-1; however, the

importance of WRN- or BLM-catalyzed strand annealing in this pathway remains to be shown. Thus, the ability of a WRN C-terminal domain that lacks the nuclease or helicase functions to rescue the replication defects of a *dna2* mutant may reflect its physical and functional interaction with FEN-1 (71), its strand-annealing activity if it is intrinsic to the C-terminal domain and active in this fragment, or a combination of both FEN-1 protein interaction and strand annealing all mediated by the WRN C-terminal domain. Alleles of *WRN* that cause WS appear to be null (7), precluding the identification of a single protein domain important for causing the disease symptoms.

Some insight as to how the coordinate functions of DNA unwinding and strand annealing by the same protein are important in a single pathway may be garnered from a model proposed for the role of *Drosophila* BLM in the synthesis-dependent strand annealing (SDSA) pathway during DSBR (72,73). According to this model, repair synthesis during SDSA is not processive, releasing the newly synthesized strand to search for a complementary sequence. In the absence of a complementary single strand, the nascent strand is further extended by a subsequent round of strand invasion and DNA synthesis. *In vitro*, several human RecQ helicases have been shown to release the invading third strand from D-loop structures (14), suggesting that the enzyme may act to release the newly synthesized strand from a RAD51-mediated D-loop formed *in vivo* during recombination or processing of replication intermediates. However, the ability of a RecQ helicase to catalyze DNA strand annealing could be utilized to anneal a newly synthesized strand to its complementary strand as the final step after repair synthesis. Unlike a HR pathway that involves HJ resolution to produce crossovers, repair of DSBs by SDSA results in gene conversion without crossing over to preserve genomic integrity.

Lastly, the coordinate action of DNA unwinding and strand annealing catalyzed by a RecQ helicase could be used for the branch migration activity on D-loop or HJ structures during HR associated with DNA repair or replication, as discussed above. Since branch migration activity requires the simultaneous disruption and annealing of complementary DNA strands, a RecQ helicase capable of catalyzing the dual activities may be well suited to fulfill this role. As suggested earlier, such coordination may help explain the ability of WRN (33) and BLM (74) to catalyze branch migration unidirectionally through long stretches of DNA.

Collaboration between RecQ helicases and PARP-1

Poly (ADP) ribose polymerase-1 (PARP-1) is a central enzyme in the DNA damage signaling process (75). Upon breaks in DNA the enzyme becomes auto-ribosylated and PARP-1 subsequently ribosylates a large number of other cellular proteins including histones. PARP-1 plays important roles in the cellular response to DNA damage including the post-translational modification of DNA repair proteins involved in repair pathways linked to the prevention of aging. Specifically, PARP-1 plays a major role in the process of BER.

It is therefore of interest that PARP interacts with RecQ helicases such as WRN. Through its physical interaction with WRN, PARP-1 modulates WRN exonuclease and helicase activities (76). Simultaneously, WRN function is required for PARP-1 ribosylation as this process is significantly diminished in WRN-deficient cells (77). Genetic cooperation between WRN and PARP-1 was demonstrated in mice whereby WRN and PARP-1 collaborate to prevent chromatid breaks, complex chromosomal rearrangements and cancer (78). There is also evidence for interaction of other RecQ helicases with PARP-1 as in the case of its association with RTS (79).

Interactions of RecQ helicases with HR proteins

RecQ helicases appear to exert a major function in DSBR. This has been demonstrated in many ways and at several levels. A number of examples of interactions between the key recombination proteins and the RecQ helicases exist (Table 2). Rad51 is a key player in the strand invasion event of HR, and this protein associates with WRN (80), BLM (81) and RTS (82). Rad51D physically interacts with BLM and stimulates BLM branch migration activity on HJ structures (83). Rad52 both inhibits and enhances WRN helicase activity in a DNA structure-dependent manner, whereas WRN increases the efficiency of Rad52-mediated strand annealing (84), suggesting they may act together in replication fork rescue after DNA damage. Rad54, another key protein in this pathway, co-localizes strongly with WRN, in particular after cellular stress (80). Linkage of WRN with the Mre11-Rad50-NBS1 complex via NBS1 (85) and the tumor suppressor BRCA1 (86) have been established. The biochemical interactions are supported by various genetic and cell biological observations demonstrating a central role for RecQ helicases as regulators of genetic recombination at stalled replication forks or in DSBR through their interactions with HR proteins.

WRN exonuclease and interactions with Ku complex

WRN is unique among the human RecQ helicases in that the protein catalyzes 3′ to 5′ exonucleolytic digestion of DNA (87–89). WRN exonuclease is stimulated by Zn^{2+} (25), consistent with the metal-binding site in the solved structure of a WRN exonuclease domain fragment (90). WRN, like other proofreading nucleases with which it shares homology (14), may remove a mismatched nucleotide incorporated by a DNA polymerase during DNA replication (88) or a DNA repair process such as BER (91,92) (see Figures 2H–I). Interestingly, although WRN interacts with human DNA polymerase δ (93) and stimulates the extension activity of yeast DNA polymerase δ (94) and human translesion DNA polymerases (95), WRN increases the amount of errors generated by Polη (95). Also, WRN exonuclease is blocked at certain lesions in DNA (96). Further work is needed to sort out the details of WRN function at a mismatch or related lesion in DNA.

WRN exonuclease activity is strongly stimulated by the Ku heterodimer (97,98), a central player in the NHEJ
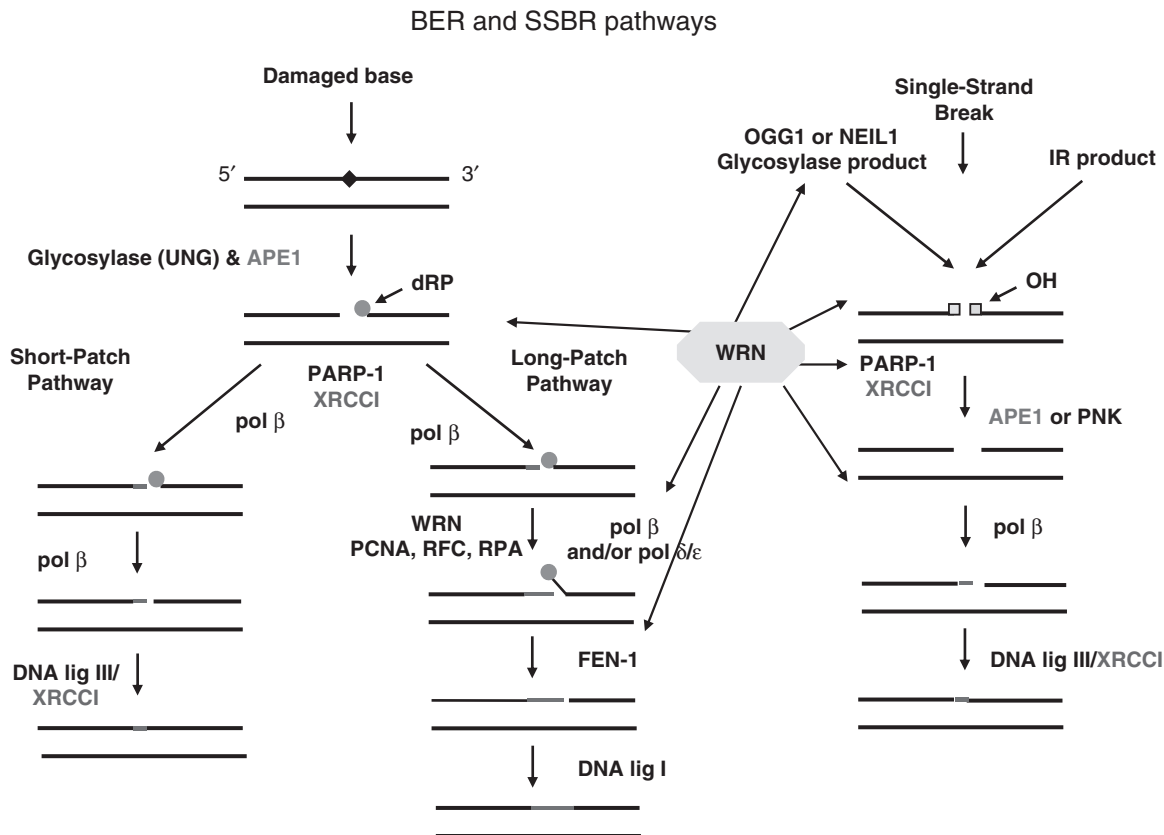
BER and SSBR pathways

Figure 3. Molecular steps and proteins of base excision repair pathways.

repair of DNA DSBs (99). It has been proposed that WRN may be a partner in the end-joining process, supported by the notion that both exonuclease and helicase activities are required in this process. However, Chen and coworkers reported that a WRN double mutant that is inactive as a helicase and exonuclease complemented the NHEJ defect of $WRN^{-/-}$ cells, suggesting a structural role of WRN in optimizing this type of DNA repair (100). WS cells do not show particularly strong sensitivity to γ-irradiation, which creates DSBs that are removed by end joining. However, it is possible that WRN is involved in this process at certain genomic sites or regions rather than in the genome overall. One such region might be the telomere, where WRN (101) and the Ku heterodimer (99) are particularly prominent.

Evidence for a role of WRN helicase in BER

The molecular steps and the proteins involved in SP-BER, LP-BER and ssDNA break repair (SSBR) are summarized in Figure 3. Notably, WRN and BLM interact functionally with many proteins involved in BER and SSBR. In particular, WRN interacts with apurinic/apyrimidinic endonuclease (APE-1), the endonuclease that incises abasic sites following lesion removal by upstream DNA glycosylases. Recent studies show that APE-1 inhibits WRN helicase (102). At the next steps in SP- and LP-BER, WRN and BLM stimulate DNA polymerase β, enhancing base incorporation (103). WRN (67) and

BLM (68) also strongly stimulate FEN-1, which cleaves 5′ protruding flaps generated by strand displacement DNA synthesis during LP-BER. *In vitro* evidence also indicates that WRN exonuclease acts as a proofreading enzyme for DNA polymerase β during LP-BER (91,92). Thus, these data indicate that WRN is an important player in BER. Although WRN is not considered to be an essential player in BER, an important role for WRN in BER is supported by the finding that WRN-deficient cells accumulate 8-oxoG (104) and are sensitive to some DNA damaging agents that are repaired by BER (91,105). While WRN interacts functionally with DNA polymerase ß and APE-1 endonuclease, it does not appear to interact with the glycosylase associated with that pathway, oxoguanine DNA glycosylase (OGG1). It does, however, interact with another glycosylase, endonuclease VIII (Nei)-like protein (NEIL1), which is stimulated by WRN (106). NEIL1 recognizes FaPy lesions in DNA and is involved in the SSBR pathway involving polynucleotide kinase (PNK). Thus, WRN participates in more than one SSBR pathway. WS cells also display a hyperoxidation phenotype (107) and rapid accumulation of protein carbonyls (5), a protein modification that is known to accumulate in older normal individuals.

Maintenance of telomere stability by RecQ helicases

Telomeres are specialized nucleoprotein complexes that reside at the physical ends of linear eukaryotic

chromosomes that exist in all vertebrates [for review, see (108)]. In most organisms studied, telomeres contain lengthy stretches of tandemly repeated simple DNA sequences composed of a G-rich strand and a C-rich strand (called 'terminal repeats'). These terminal repeats are highly conserved since all vertebrates appear to have the same telomeric DNA repeat of (TTAGGG)n which repeats for several kilo-bases. These tandem repeats form a scaffold that many different telomeric proteins bind forming the DNA-protein complex (109). Telomeric DNA has a variety of structures within it [for review, see (110)]. The 3′ single-stranded DNA tail is thought to play an important role in the structure and function of telomeres (111,112). In certain cells that utilize an alternative lengthening of telomere (ALT) pathway, the 3′ end mediates the formation of large loops where the presence of the 3′ G strand overhang at the end of the telomere is tucked back inside the double-stranded DNA at the loop junction, forming a structure referred to as the *t*-loop (113). The area where the 3′ G strand extension invades the duplex telomeric repeats is called 'D loop' (displacement loop). Given the fact that the 3′ tail extension is G-rich, another structure that may arise during telomere metabolism is the tetraplex stabilized by planar arrays of four hydrogen-bonded guanines (114). Evidence in support of this was provided by the first crystal structure of quadruplexes formed from human telomere repeat sequences (115). *In vivo* evidence for the existence of G-quadruplexes at telomeres (116) and other regions of the genome (117) was provided by cellular and molecular studies. For further reading on the experimental evidence pertaining to the existence and dynamic role of G4 DNA in living cells, see (117).

The telomere caps function to protect the gene containing regions of the chromosome from events that may lead to genome instability. In the absence of functional telomeres, eukaryotic chromosomes become unstable (118–120). The ends of the chromosomes may be mistaken for DSBs by the DNA repair machinery. This may lead to events that include degradation of the terminal region of the chromosome, inappropriate recombination or fusion of the ends of two chromosomes. All of these processes would lead to abnormal chromosomes that would break during mitosis, and result in severe damage to the genome and the activation of DNA damage checkpoints. Ultimately, this may give rise to activation of apoptotic pathways or cellular senescence (121).

Telomere-specific proteins that are associated with the human TTAGGG repeats at the ends of human chromosomes and protect chromosome ends are referred to collectively as a protein complex called 'shelterin'. Currently, six shelterin subunits have been identified: TRF1, TRF2, TIN2, Rap1, TPP1 and POT1. TRF1, TRF2 and POT1 directly recognize and bind to the TTAGGG repeats, and in conjunction with the TIN2, Rap1 and TPP1 proteins form a protein complex that distinguishes telomeres from other sites of DNA damage. The shelterin complex protects telomeres by directly affecting the structure of telomeric DNA [for an in-depth review, please see (122)]. In addition to the shelterin

Table 3. Molecular interactions of WRN with telosome proteins and telomeric DNA structures

Direct WRN-interacting shelterin proteins	
TRF1	
TRF2	
POT1	
Ku 70/80	
Telomere-interacting protein partners of WRN	
ATM	Rad51/Rad52
NBS1	RPA
BLM	p53
PARP-1	
WRN-interacting telomeric DNA structures	
3′ ssDNA overhang	
T-loop	
G4 tetraplex	
Holliday junction	

proteins, a number of 'tool-belt' DNA processing factors (Mre11/Rad50/Nbs1, ERCC1/XPF, DNA-PK, PARP-2, Tankyrases and RAD51D) affect telomere metabolism. Together the shelterin and non-shelterin proteins have important functions in the protection, replication and stabilization of the chromosome ends. Several lines of evidence have also implicated certain RecQ helicases in telomere maintenance. The *S. cerevisiae* Sgs1 helicase participates in a Rad52-dependent ALT pathway that operates in telomerase negative mutants (123). Expression of catalytically active human BLM helicase rescued the telomere defects in telomerase-negative *sgs1* (124), suggesting that BLM and Sgs1 act catalytically upon similar telomeric structures. BLM was observed to promote telomeric DNA synthesis in ALT cells (125) and interacts with the telomeric repeat-binding factors TRF1 and TRF2 (126,127).

Molecular interactions of WRN protein with a number of telosome proteins and telomeric DNA structures have been reported (Table 3), and WRN helicase-nuclease is implicated in telomere maintenance from a number of biochemical and biological studies. Stochastic loss of telomeres has been reported in cells deficient for WRN protein. WS fibroblasts display defects associated with telomere dysfunction, including accelerated telomere erosion and premature senescence (128–130). The premature senescent phenotype of WS fibroblasts can be rescued by forced telomerase expression (131). Over-expression of telomerase restores genomic stability to WS fibroblasts (132). Single telomere length analyses suggested similar rates of telomere erosion in HPV E6-expression clones of WS cells compared to normal cells; however, in bulk cultures, even with single telomere measurements, the rate of telomere shortening was greater in WS cells (133). Expression of dominant-negative WRN in a human tumor cell line expressing telomerase resulted in an increased telomere loss and chromosome function (134), suggesting a telomerase-independent function of WRN in telomere length maintenance that is consistent with the role of Sgs1 in an ALT pathway for telomere maintenance via HR.

A role for WRN in proper capping of telomeres has been proposed (135). Biochemical studies suggest that WRN helicase collaborates with proteins of the shelterin complex to facilitate telomere maintenance (Table 3). For example, DNA unwinding catalyzed by WRN (and BLM) helicases is stimulated by human TRF2 (125,127,136). WRN promotes unwinding of model D-loop structures containing telomeric sequences (Figure 2F) and is present at telomeres along with other DNA repair proteins in cells that employ the ALT pathway (101), suggesting a role of WRN helicase to facilitate the ALT pathway. Interestingly, both catalytic activities (helicase and exonuclease) of WRN are involved in the resolution of a D-loop substrate (101) (Figure 2F). WRN exonuclease needs to digest from the 3′ end in order to decrease the duplex length, and thereby allows WRN helicase to unwind the remaining duplex region in the absence of RPA. The ability of WRN and BLM helicases to unwind telomeric DNA structures is stimulated by the telomeric SSB POT1 (protection of telomeres-1) (137). Altogether, the results are consistent with a model in which telomere-binding factors co-operate with mammalian RecQ helicases in resolving telomeric DNA structures in a manner that protects the telomeric 3′ tail as it is exposed during unwinding. Furthermore, WRN is necessary for efficient replication of G-rich telomeric DNA, and defective telomere lagging strand synthesis was reported in cells lacking WRN helicase activity (138).

Curiously, a disease phenotype in WRN-deficient mice is absent (139) (Table 1). However, the long telomeres of mice may have a protective effect in WS pathogenesis. In support of this idea, late-generation telomerase and WRN-deficient mice ($mTerc^{-/-}$ $WRN^{-/-}$) with dysfunctional telomeres, generated independently by two laboratories, display clinical symptoms resembling WS (140,141). The $mTerc^{-/-}$ $WRN^{-/-}$ mice were characterized by many of the clinical symptoms of premature aging and the types of tumours (osteocarcinomas, soft tissue sarcomas) typically observed in WS patients. Cells from the $mTerc^{-/-}$ $WRN^{-/-}$ mice displayed accelerated telomere shortening, chromosomal instability and replicative senescence. The $mTerc^{-/-}$ $WRN^{-/-}$ cells were also shown to have elevated recombination rates between telomeres of sister chromatids (142). Restoration of normal telomere SCE was dependent on WRN helicase activity. The *WRN*-deficient telomere dysfunctional cells escaped senescence by the ALT pathway. These findings suggest that the chromosomal instability and cancer of WS is a consequence of aberrant telomere SCE that activates the ALT pathway [for a review, see (143)].

Similar to $mTerc^{-/-}$ $WRN^{-/-}$ mice, the pathology of later-generation mice with an $mTerc^{-/-}$ allele combined with a hypomorphic BLM^{M3} allele showed an acceleration of phenotypes characteristic of later-generation *Terc* mutants (141) (Table 1). Pathologies not observed in *Terc* mutants, such as bone loss observed in WS and BS, were evident in $mTerc^{-/-}$ double mutant backgrounds with mutations in *WRN* or *BLM*. The phenotypes associated with later-generation mice were more severe in $mTerc^{-/-}$ $WRN^{-/-}$ $BLM^{-/-}$ triple mutants than the sum of these defects in each single mutant, suggesting a synergistic interaction of WRN and BLM helicases in telomere biology. Thus, the delayed manifestation of the complex pleiotropic phenotypes of *WRN* or *BLM* deficiency in mice may relate to telomere shortening.

Resolution of alternate DNA structures by RecQ helicases

Motifs for the formation of G-quadruplex DNA structures are widely dispersed in eukaryotic genomes, and can be found in telomeres, gene promoters elements and recombination hotspots (144). Cellular pathways including replication, recombination, transcription and telomeric DNA elongation involve steps in which two strands of duplex DNA can be unwound transiently, providing an opportunity for the G-rich strand to form quadruplex structures (145). Unregulated formation of G4 DNA within chromosomal regions may potentially lead to genomic instability or interfere with fundamental processes of nucleic acid metabolism, including replication or transcription.

RecQ family members are unusual in that they preferentially unwind tetraplex DNA (Figure 2E) and other alternate DNA structures (triplex DNA, synthetic D-loops and HJs) compared to B-form DNA (74,146–151). Both yeast Sgs1 helicase and its close human homolog, BLM, unwind G4 DNA with at least 15-fold preference relative to duplex substrates (148,150). Furthermore, both BLM and Sgs1 preferentially unwind G4 DNA relative to HJ substrates, reflecting an increased G4 binding affinity (152). The G4 binding domain of BLM and *E. coli* RecQ was mapped to the conserved RQC region of the proteins (153). G4 DNA unwinding activity is proposed to contribute to the function of Sgs1 in the maintenance of two G-rich genomic domains, ribosomal DNA (154,155) and telomeres (156–158). A role of Sgs1 in ribosomal DNA metabolism is suggested by the observations that *sgs1*-deficient strains are characterized by nucleolar fragmentation (159,160). Like Sgs1, WRN is also suspected to have a role in ribosomal DNA metabolism based on the observation that a significant fraction of WRN is nucleolar (161). Based on *in vivo* evidence, it was proposed that WRN helicase insures efficient replication of G-rich telomeric DNA, resulting in the proper maintenance of telomeres and genomic stability (138). G4 tetraplex resolution by WRN helicase was proposed to be required for telomeric DNA replication since S-phase-dependent telomere loss was detected in the absence of WRN. Moreover, WRN depletion affected replication of the telomeric G-rich lagging strand template that is proposed to form G4 structures (138). WRN helicase facilitates yeast pol δ synthesis through tetraplex and hairpin structures (162), suggesting a role of WRN in disruption of DNA roadblocks that impede replication (Figure 2D–E). BLM and WRN helicases also resolve triple helix structures that may interfere with replication or transcription (146).

Interactions between RecQ helicases

In yeast there is only one RecQ helicase, Sgs1 (*S. cerevisiae*) or Rqh1 (S. *pombe*), whereas in humans, there are five. It is of great interest to better understand why so many RecQ helicases are required in humans and how they interact or interplay in the different pathways in which they operate. How much redundancy between the RecQ helicases exists? Can one RecQ helicase take over for another? Studies addressing these questions have been performed at various levels and in different biological systems. At the biochemical level, there is evidence for a functional protein interaction between WRN and BLM helicases (163), as BLM inhibits WRN exonuclease. This is also supported by observation of colocalization between these helicases, in particular after cellular stress (163). As yet there is no evidence for any synergistic function between these RecQ helicases, but it seems a likely possibility, worthy of future investigation, to determine whether there may be a specific substrate, for example, a DNA modification, on which these helicases can together accomplish a function that neither of them can do alone.

Functional roles of RECQ1 and RECQ5

RecQ helicases have multiple roles in DNA repair, replication and recombination, S-phase checkpoint and telomere maintenance; consequently, they are considered caretakers of the genome (14,164). Three of the five human RecQ genes, designated *BLM*, *WRN* and *RECQ4*, have been genetically linked to the autosomal recessive diseases BS, WS and RTS, respectively, characterized by a predisposition to cancer and chromosome instability, but the clinical features and cellular phenotypes are different from each other, suggesting unique roles of BLM, WRN and RECQ4 helicases as tumor suppressors. For a recent review on the molecular genetics of RECQ4, please see (165).

The biological significance of the remaining two human RecQ helicases, RECQ1 and RECQ5, is not yet known. Very little is known about the cellular functions of RECQ5 [for review, see (8)]; consequently, the focus of this section of the review will primarily be on RECQ1. The *RECQ1* (*RECQL*) gene, originally cloned independently by two groups (166,167), encodes the most abundant of the five human RecQ helicases in resting B cells, and its expression is upregulated in response to EBV transformation or treatment with the tumor-promoting agent, phorbol myristic acetate (168). Despite the fact that RECQ1 was the first human RecQ helicase protein to be identified, little is known about its genetic functions in mammalian cells. Studies utilizing chicken DT40 cells have shown that RECQ1 and RECQ5 have roles in cell viability under a BLM-impaired condition, indicating a backup function for these helicases (56). However, BLM, RECQ5 and RECQL have non-redundant roles in suppressing crossovers in mouse ES cells and fibroblasts (57,58) (Table 1), suggesting a cell type or species–specific difference between the chicken and the mouse systems.

RECQL-deficient mice do not exhibit any apparent phenotypic differences when compared to wild type mice and appear to be normal and fertile, with normal telomere lengths. Cytogenetic analyses of embryonic fibroblasts from the RECQL-deficient mice revealed aneuploidy, spontaneous chromosomal breakage and frequent translocation events. RECQL-deficient cells were also hypersensitive to ionizing radiation and exhibited an increased load of DNA damage, suggesting that RECQL has a unique cellular role in DNA repair required for genomic stability. Genetic background, functional redundancy and perhaps other factors may protect the unstressed mouse from the types of abnormalities that might be expected from the severe chromosomal aberrations detected at the cellular level. It is conceivable that manifestation of cellular or organismal phenotypes in *Recql*-null mice may be masked by other genetic factors. For example, *WRN*-null mice do not exhibit any phenotypes prevalent in WS; however, premature aging phenotypes are observed in a *WRN*-null telomerase-knockout mouse characterized by the presence of critically short telomeres [for review, see (135)]. Furthermore, late generation $mTerc^{-/-}$ $WRN^{-/-}$ mouse embryonic fibroblasts have an increased level of DNA damage markers and replicative senescence, properties similar to that observed in human WS fibroblasts.

Although a genetic disorder has not yet been linked to a mutation in RECQ1, recent analyses of *RECQ1* single nucleotide polymorphisms have identified an association of RECQ1 with a reduced survival of pancreatic cancer patients (169,170). The chromosomal instability arising from RECQ1 deficiency may contribute to a cancer predisposition. Consistent with this notion, RECQ1 is differentially upregulated in transformed cells or cells that are actively proliferating (168).

Very recently, RECQ1 was identified as a member of a piRNA protein complex that is important for gene silencing (171). This is an unexpected and potentially exciting role for RECQ1 protein in the metabolism of regulatory small RNAs important for development and mechanisms of inheritance. The mechanistic similarities between piRNA synthesis and DNA replication (172) suggest that RECQ1 and its associated DNA replication/repair proteins [RPA (29), EXO-1 and MSH2/6 (173)] may conceivably function together involved in piRNA biogenesis. Further work is required to understand RECQ1-piRNA genome defense association. Presently, it can only be speculated if the piRNA complex regulates the genome at the level of DNA or histones, or at a post-transcriptional level. Interestingly, QDE-3, the RECQ1 homolog in *Neurospora crassa*, has also been implicated in gene silencing (174). The *qde-3* mutant was found to be hypersensitive to a variety of DNA mutagens and exhibit increased mutability and extensive chromosomal deletions (175–177), implicating a broader role of the *Neurospora* RECQ1 homolog in the DNA damage response and chromosomal stability. A high priority will be to define the mechanism(s) of RECQ1 in its inter-connected roles in gene silencing and chromosomal stability maintenance.

Post-translational modifications of RecQ helicases have functional implications

There is a growing interest in the molecular and biological consequences of post-translational modifications of RecQ helicases. One of the well-characterized protein modifications of a mammalian RecQ helicase is WRN phosphorylation (12). WRN becomes phosphorylated after cellular exposure to the double-strand breaking agent bleomycin or after replicational stress (178–181). Protein kinases responsible for WRN phosphorylation are DNA-PKcs, ATM and c-Abl. WRN phosphorylation by DNA-PK complex inhibits WRN exonuclease and helicase activities and may be important in the NHEJ pathway of DSBR (179). c-Abl phosphorylation of WRN protein, also influenced by cellular exposure to DNA damaging agents such as bleomycin, inhibits WRN helicase and exonuclease activities as well (178). The precise pathways of the DNA damage response that are regulated by phosphorylation of WRN and other RecQ helicases remain to be characterized. BLM is likely phosphorylated by ATM in response to ionizing radiation and ATR in response to DNA replicational stress [for review, see (8)].

Other types of known WRN protein post-translational modifications include acetylation and sumoylation. It has been proposed that SUMO modification of WRN is important for sub-nuclear relocalization of WRN during the DNA damage response (182,183). Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is also regulated by SUMO modification (184). p300 acetylation of WRN also affects its sub-nuclear relocalization after DNA damage (185). Understanding the consequences of protein post-translational modifications for RecQ relocalization and function in the response to blocked or damaged replication forks, DSBs directly introduced by DNA damaging agents, and other types of DNA base modifications will be important to assemble regulatory pathway models that will hopefully lead to greater insight of the deficiencies associated with diseases of genomic instability, cancer and aging.

Therapeutic implications of RecQ helicase research

RecQ helicase disorders provide provocative models that are useful for studying human diseases of aging and cancer. Although the RecQ helicase disorders identified are relatively rare, the mutational status of RecQ helicase genes may have pathological influences that are yet to be fully appreciated. For example, the possibility that heterozygous mutations in the *WRN* gene may predispose individuals to age-associated symptoms has attracted interest (7). An ongoing effort has been the molecular diagnosis of WS and clinical outcomes [The International Registry of Werner syndrome (www.wernersyndrome.org)] (9). Only a limited number of WRN polymorphic variants existing in the normal population that potentially affect aging have been studied (186,187). Clearly, much work is to be done to identify and characterize polymorphisms in RecQ helicases as risk factors for age-related diseases or cancer.

Several human RecQ helicases have been shown to be upregulated in rapidly proliferating or transformed cells (168). Because of their essential roles in DNA metabolism, and more specifically the DNA damage response, dysregulation of RecQ helicases may be a central feature in aging and cancer, more specifically in age-associated diseases. Thus treatment affecting the functions of RecQ helicases could be of great relevance to cancer (188–190) and other diseases. Combinatorial treatments have been proposed for combating cancer by inhibiting RecQ helicase function in tumor cells that already have compromised DNA repair and/or DNA damage signaling. Of particular interest is the possibility that DNA helicases such as WRN may be targeted to impair DNA cross-link repair or DSBR since a number of DNA chemotherapy strategies rely on radiation or DNA damaging drugs that introduce interstrand cross-links or double-strand breaks. The idea that WRN helicase may be a suitable target for chemotherapy treatments is supported by the observation that epigenetic inactivation of the *WRN* gene by hypermethylation in colorectal tumors served as a useful predictor of robust clinical response to irinotecan, a topoisomerase inhibitor used to clinically treat this tumor type (191).

The abundant evidence for the direct involvement of RecQ helicases in either NHEJ or HR pathways of DSBR provides a strong incentive to screen small molecule libraries to identify inhibitors of DNA helicases such as WRN or BLM. These compounds might be tested in model systems for cytotoxicity in combination with various DNA damaging agents, replication inhibitors or radiation to identify viable combinatorial strategies. Characterization of small molecule RecQ helicase inhibitors in such model systems may lead to their implementation in preclinical and clinical studies.

As discussed above, the WRN and BLM helicases efficiently resolve G4 tetraplex structures. A number of DNA binding compounds that preferentially interact with G4 DNA have been investigated as potential anticancer drugs since G-rich DNA is found in telomeres, gene promoter elements such as the *c-MYC* oncogene and recombination hotspots (144). Notably, MYC stimulates expression of the *WRN* gene and WRN upregulation promotes MYC-driven tumorigenesis by preventing cellular senescence (192,193). Inhibition of WRN or BLM unwinding of tetraplex structures by small molecules that stabilize G4 DNA may be used to regulate gene expression of oncogenes or affect telomere metabolism. Malignant transformation is associated with the upregulation of telomerase expression and telomerase activation plays a role in tumor formation (194). As an alternative to inhibition of telomerase expression or activity, G4 structures at telomeres may be stabilized by G4-interactive small molecules that prevent access of telomerase to the telomeric ssDNA. Inhibition of G4 resolving helicases such as WRN or BLM may provide yet another strategy to inhibit telomerase function by preventing access of telomerase to the ssDNA template.

Although RecQ helicases represent a viable target for anti-cancer therapy, potential obstacles include the non-specific cytotoxicity of DNA damaging treatments

with helicase inhibitors, therapy-related carcinogenesis and the potential functional redundancy of DNA damage response helicases. These topics are discussed in (189). Despite these caveats, RecQ helicases are a unique class of DNA repair proteins that may serve as viable targets to chemosensitize cancer cells.

Perspectives

Understanding the mechanistic basis of human aging is a formidable challenge. The connection of age-related diseases and cancer to mutations in genes that control chromosomal stability provide an opportunity to study genetic, molecular, cellular and ultimately organismal aspects of the aging process. In this regard, study of the RecQ family of DNA helicases has provided new insights into the critical importance of genome integrity and DNA repair for genome homeostasis and the prevention of cellular decline in function. The importance of RecQ helicases is emphasized by their direct roles as guardians of the genome. An even more in-depth understanding of the mechanisms of RecQ helicases in pathways of DNA metabolism and maintenance of genomic stability during the next decade will surely lead to new advances in aging and cancer research since the cloning of the first human RecQ helicase genes *WRN* and *BLM*, a little over a decade ago. Excitement abounds in the RecQ helicase field for the discoveries which lie ahead that may lead to better diagnosis, treatment, and understanding of cancer and aging.

ACKNOWLEDGEMENTS

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