The RecQ DNA Helicases in DNA Repair

Kara A. Bernstein,[1] Serge Gangloff,[2,3] and Rodney Rothstein[1]

[1]Columbia University Medical Center, Department of Genetics & Development, New York, New York 10032; email: kab2142@columbia.edu, rothstein@cancercenter.columbia.edu

[2]CEA, DSV, iRCM, SIGRR, LERA, Fontenay-aux-Roses F-92265, France; email: serge.gangloff@cea.fr

[3]CNRS, UMR 217, Fontenay-aux-Roses F-92265, France

Annu. Rev. Genet. 2010. 44:393–417

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi: 10.1146/annurev-genet-102209-163602

0066-4197/10/1201-0393$20.00

Key Words

RecQ, Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome, homologous recombination, DNA repair

Abstract

The RecQ helicases are conserved from bacteria to humans and play a critical role in genome stability. In humans, loss of RecQ gene function is associated with cancer predisposition and/or premature aging. Recent experiments have shown that the RecQ helicases function during distinct steps during DNA repair; DNA end resection, displacement-loop (D-loop) processing, branch migration, and resolution of double Holliday junctions (dHJs). RecQ function in these different processing steps has important implications for its role in repair of double-strand breaks (DSBs) that occur during DNA replication and meiosis, as well as at specific genomic loci such as telomeres.

INTRODUCTION

Double-strand break (DSB): DNA damage that results in a break of both strands of DNA

DNA topoisomerases: enzymes that alter DNA topology by catalyzing strand passage

DNA helicase: an enzyme that unwinds complementary duplex DNA

The failure to repair DNA damage can lead to genomic instability. Repair of double-strand breaks (DSBs) requires many proteins with enzymatic activities, such as nucleases, helicases, ligases, DNA topoisomerases, etc. One important family of enzymes is DNA helicases, and these enzymes function by unwinding complementary strands of DNA. The RecQ DNA helicases, conserved from bacteria to humans (**Figure 1**), are critical to ensure proper repair of DNA damage. Bacteria and budding yeast have one RecQ homolog, RecQ and Sgs1, respectively. In humans, there are five RecQ homologs, and mutations in three of these genes (BLM, WRN, RTS/RECQ4) are associated with Bloom, Werner, and Rothmund-Thomson syndromes, respectively, which cause cancer predisposition and/or premature aging (44, 79, 121, 131, 169).

Unwinding of double-stranded DNA (dsDNA) is necessary in many different processing steps. One of the challenges in studying the function of the RecQ helicases is that mutation of the RECQ genes leads to a pleiotropic phenotype, exhibiting traits that include increased chromosomal rearrangements, increased sister-chromatid exchanges (SCEs), and premature aging. The RecQ helicases function in both early and late recombination steps, where they promote homologous recombination (HR) and prevent crossover events. This review focuses on the recent findings that the RecQ proteins function at multiple DNA processing steps and how these

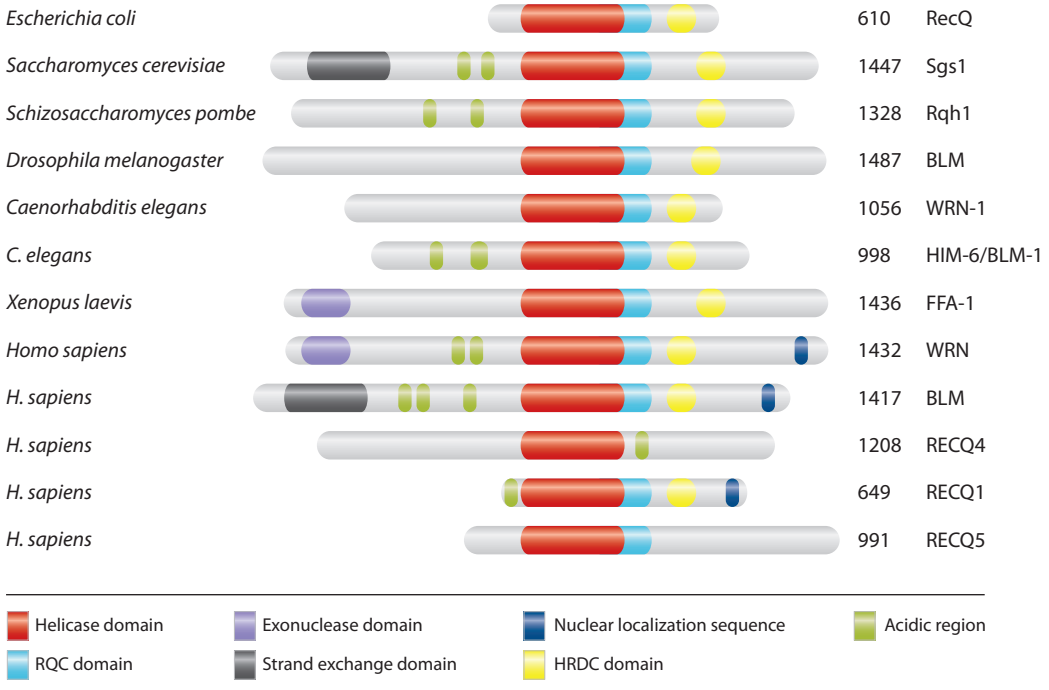

Figure 1

Structural features of RecQ helicases. The RecQ proteins have several structural domains that are conserved from bacteria through humans. All RecQ proteins have a core helicase domain. Most RecQ proteins also contain conserved helicase and RNAse D C-terminal (HRDC) and RecQ C-terminal (RQC) domains that are thought to mediate interactions with nucleic acid and other proteins, respectively. Many RecQ proteins have acidic regions that enable protein-protein interactions, and some of the RecQ proteins have nuclear localization sequences. WRN and FFA-1 protein are unique in that they also contain an exonuclease domain. Sgs1 and Blm are the first characterized members of this family of proteins containing a functional strand exchange domain in their N-terminus. The number of amino acids in each protein is indicated on the right.

Table 1 Clinical features of RecQ disorders

Syndrome (gene)	Main clinical features	Cancer predisposition
Bloom syndrome (BLM)	Dwarfism, beaked nose, narrow face, pigmentation, redness, and dilated blood vessels in skin, mental retardation, type-II diabetes, immunodeficiency, lung problems, low or no fertility	Early onset with normal distribution of tissue and type
Werner syndrome (WRN)	Bilateral cataracts, hoarseness, skin alterations, thin limbs, premature gray/loss of hair, pinched facial features, short stature, osteoporosis, hypogonadism, diabetes, soft tissue calcification	Early onset of primary sarcomas and mesenchymal tumors
Rothmund-Thomson syndrome (RECQ4)	Poikiloderma, juvenile cataracts, growth retardation, skeletal dysplasia, sparse scalp hair, hypogonadism	Early onset of osteosarcomas
RAPADILINO syndrome (RECQ4)	Growth retardation, bone malformation in limbs, radial defects such as hypoplasia and aplasia of thumbs and radius, cleft or highly arched palate	Lymphoma and osteosarcoma
Baller-Gerold syndrome (RECQ4)	Craniosynostosis, radial aplasia and hypoplasia, poikiloderma, growth retardation	Not applicable

functions enable these proteins to act on many different biological substrates. Furthermore, we highlight the results from the budding yeast RecQ, protein Sgs1, and human homologous BLM and WRN as models of RecQ function because these are the most extensively studied RecQ proteins.

RECQ AND HUMAN DISEASE

In humans, mutations in BLM, WRN, or RECQ4 lead to separate genetic diseases, Bloom, Werner, and Rothmund-Thomson syndromes, respectively (130) (**Table 1**). The other two RecQ helicases, RECQ1/RECQL1 and RECQL5, have not been associated with heritable diseases. However, a single nucleotide polymorphism in RECQ1 correlates with decreased survival of pancreatic cancer patients (91), and *Recql5*$^{-/-}$ knockout mice also display increased cancer rates (65).

Bloom Syndrome

Bloom syndrome is a rare autosomal recessive genetic disorder characterized by growth retardation, light sensitivity, immunodeficiency, male infertility, and increased cancer (14, 130) (**Table 1**). The predisposition of Bloom patients to develop all types of cancers, frequently occurring by the fourth decade of life, is the primary cause of death. This disease is due to mutations in the *BLM* gene, and there are approximately 230 documented cases of Bloom syndrome patients (44, 53). From the extensive mutational analysis of the *BLM* gene in these patients, it was concluded that this disease likely originated with a small number of founder mutations (53). The increased cancer incidence correlates with chromosomal breaks and SCEs, both of which are increased in Bloom patient cells (52).

Mouse models of Bloom syndrome have been created to mimic the phenotype observed in humans with this disease. Blm is expressed during embryogenesis in mice in most tissue types, with highest expression in the spleen, thymus, testis, and ovaries (33). Disruption of the *Blm* gene by insertion of a neomycin cassette in the region upstream to the helicase domain leads to embryonic lethality at 13.5 days gestation in homozygous mutant mice, suggesting that in mice Blm expression is essential during embryogenesis (33). When embryos are analyzed at 9.5 days postconception (dpc), *Blm*$^{-/-}$ mice are 50% smaller than wild-type or heterozygous *Blm*$^{+/-}$ mice. This size discrepancy continues until death at 13.5 dpc, indicating that, like humans with Bloom disease, *Blm*$^{-/-}$ mice also display growth retardation (33). Also consistent with the human disease, embryonic fibroblasts derived from

Double-stranded DNA (dsDNA): two base-paired complementary strands of DNA

Sister-chromatid exchange (SCE): reciprocal recombination between two sister chromatids

Homologous recombination (HR): a DNA repair mechanism that utilizes a homologous sequence as the template for repair

Loss-of-heterozygosity: deletion, mutation, or recombination events that result in loss of the wild-type allele in a heterozygote

Telomeres: sequences that protect the ends of chromosomes from degradation and prevent the recognition of chromosome ends as DNA damage

Blm$^{-/-}$ mice display increased SCEs, which can be seen in metaphase spreads of differentially stained chromosomes (33).

To mimic more closely the mutations found in humans, another *Blm* mutant mouse was generated. This mutant mouse mimics the predominant mutation found in Ashkenazi Jews, a truncation in the helicase domain of the *Blm* gene (44, 57). In this mouse model, exons 10, 11, and 12 were replaced (44, 57). Complete disruption of exons 10, 11, and 12 leads to embryonic lethality (57). However, mice heterozygous for this truncation are more susceptible to cancer when combined with other heterozygous mutations, such as those that inactivate tumor-suppressor genes like *Apc* (anaphase promoting complex) (57). This mouse model demonstrates that haplo-insufficiency is adequate to promote tumor formation.

Additional mouse models have been created carrying mutant *Blm* alleles. One encodes a truncated protein, which is found in several Bloom patients (99). *Blm* mice with this truncation are viable and do not display growth retardation; however, they do show increased SCE (99). Unlike wild-type mice, by 20 months approximately 30% of these *Blm* mice develop cancer. Furthermore, similar to Bloom patients, the cancers observed in these mice represent a broad range (i.e., sarcomas, lymphomas, and carcinomas) (99). Consistent with increased SCE, embryonic stem (ES) cells derived from these mice have an 18-fold increase in loss-of-heterozygosity, which is one of the mechanisms that can lead to complete loss-of-function of tumor-suppressor genes in cancer (99).

Together, these mouse models demonstrate that the Blm protein normally functions by repressing SCE. In the absence of Blm protein, unregulated SCE leads to loss-of-heterozygosity, providing a mechanism to explain why Bloom patients are particularly susceptible to a wide range of cancers.

Werner Syndrome

Werner syndrome leads to premature aging, with an early onset of diseases like cataracts and osteoporosis, as well as genomic instability predisposing these patients to tumor formation (130) (**Table 1**). Interestingly, Werner patients typically exhibit normal development until adolescence and subsequently symptoms emerge in their early 20s that result in death around 46–54 years of age (58). Cells derived from Werner patients show an increased frequency of chromosomal rearrangements such as translocations, inversions, and deletions (47, 48, 129). Werner patients' tumors are distinct from those of Bloom patients, being mainly of mesenchymal origin, such as sarcomas (66). The vast majority of patients with Werner syndrome have been linked to a founder mutation in Japan (58).

Several mouse models have been created to mimic the phenotype observed in Werner patients. In one mouse model, the helicase domain was disrupted (specifically helicase domains III and IV), resulting in a truncated but stable protein (87). ES cells derived from these mice are not sensitive to many DNA damaging agents (i.e., UV, gamma irradiation, mitomycin C) but are sensitive to camptothecin, which specifically inhibits topoisomerase I (87). Disappointingly, this mutant mouse does not display the premature aging phenotype observed in Werner syndrome.

A breakthrough in recapitulating Werner syndrome in mice occurred when the effect of the *Wrn* mutation was examined in animals altered for telomere function (30). In this study, *Wrn*$^{-/-}$ mice were combined with null alleles of *Terc*, which encodes the telomerase RNA needed for telomere lengthening (30). After homozygous null mice were interbred for 4–6 generations, approximately 60% of the 14–16-week-old *Terc*$^{-/-}$ *Wrn*$^{-/-}$ mice displayed features of premature aging (i.e., hair loss, cataract formation, hypogonadism) after a normal early adulthood (30). Many of these mice also showed early-onset osteoporosis, type II diabetes, and decreased wound healing. These results demonstrate that many of the key features observed in Werner patients could be explained, at least in part, by the role of WRN in maintenance of

telomeres. Through fluorescence in situ hybridization (FISH) studies, it was shown that fused chromosome arms and shortened telomeres were present in cells derived from the *Terc*$^{-/-}$ *Wrn*$^{-/-}$ mice. Furthermore, mouse embryonic fibroblasts (MEFs) derived from these mice exhibit increased 53BP1 foci, a marker for DSB repair, and increased gamma-H2AX foci, an indicator of DSBs. Interestingly, the *Terc*$^{-/-}$ *Wrn*$^{-/-}$ mice are not cancer prone. However, after several generations of inbreeding, *Terc*$^{-/-}$ *Wrn*$^{-/-}$ mice develop many osteosarcomas and soft tissue sarcomas (30). Therefore, creation of the Werner mouse model demonstrated that, unlike the other RecQ homologs, Wrn's distinct role in telomere maintenance is likely inter-related to the clinical features observed in Werner patients.

RECQ4-Associated Diseases

Mutations in RECQ4 are associated with three unrelated disorders; Rothmund-Thomson syndrome (RTS), RAPADILINO syndrome, and Baller-Gerold syndrome (BGS) (80, 136, 137, 146) (**Table 1**). All of these disorders are characterized by growth retardation and radial defects. However, RAPADILINO syndrome patients do not exhibit poikiloderma, which is characteristic of both RTS and BGS. RAPADILINO is most prevalent in Finland, unlike the other two RECQ4 disorders (136). RTS is the best characterized of the RECQ4 diseases, and these patients also have skeletal abnormalities, skin disorders, light sensitivity, and age prematurely (80, 130, 137). Rothmund-Thomson patients are especially susceptible to developing bone and skin cancer, and their cells display increased chromosomal rearrangements like translocations and deletions (103).

Several mouse models have been created that mimic RTS. Given that most Rothmund-Thomson patients have mutations in RECQ4 that predominately map to the helicase, the mouse models have focused on this region. In one model, *Recq4* exon 13, which encodes motif III of the helicase domain, was deleted (64). Although the majority of the mice die within two weeks after birth, those that survive are smaller than wild-type mice (64). These mice also display skin abnormalities such as hair loss, graying hair, and thin, dry skin. However, unlike Rothmund-Thomson patients, these mice do not exhibit poikiloderma, osteosarcomas, and cataracts (64).

Another viable mouse model was created that more closely mimics RTS by disrupting the helicase domain encoded by exons 9 through 13 of *Recq4* (101). This disruption leads to a truncated protein due to a premature stop codon, and 16% of these mice die within 24 hours of birth. The surviving mice develop hypo/hyperpigmentation on their tails and show an increased incidence of skeletal defects in their limbs (101). Unlike RTS patients, all of these mice have palatal patterning defects and a wild-type life span. Like the *Blm* mutant mouse model, when the *Recq4* mutant is combined with a mutant *Apc* tumor suppressor gene, the double mutant mice are more likely to develop cancer (101). MEFs derived from the *Recq4* mutant mice exhibit chromosome instability resulting in aneuploidy. These mouse models for RTS demonstrate that many aspects of the phenotype observed in humans are also seen in mice with mutations in *Recq4*.

Single-stranded DNA (ssDNA): stretches of single-stranded DNA that can be coated by the replication protein A (RPA) complex or the Rad51 recombinase

STRUCTURE OF RECQ HELICASES, COMPLEX FORMATION, AND BINDING PARTNERS

RecQ helicases have been found in bacteria, fungi, animals, and plants, and their copy number ranges from one in *Escherichia coli* and *Saccharomyces cerevisiae* to up to seven in *Arabidopsis* (reviewed in 62). They all share a similar overall structural organization in which the helicase domain, containing a DEAH box, functions to unwind DNA in an ATP- and Mg^{2+}-dependent manner (**Figure 1**). RecQ helicases belong to the SF2 superfamily (56), are involved in the resolution of DNA structures, and travel on single-stranded DNA (ssDNA) in a 3′→5′ direction (reviewed in 4). The RecQ C-terminal (RQC) domain is characteristic of most RecQ helicases and is believed to mediate protein-protein interactions; however, it

is absent in RECQ4 and RECQ5. The helicase and RNase D C-terminal (HRDC) domain (80 amino acids) found at the C terminus of many RecQ helicases allows interactions with nucleic acid (95) but is absent from RECQ1, RECQ4, and RECQ5. The HRDC domain forms a structural scaffold; however, models of the HRDC domain indicate that the surface properties are not equivalent in different RecQ helicases, suggesting that they could be involved in the positioning of a specific substrate through steric interactions or secondary contacts with the DNA (95). In addition to the helicase, RQC, and HRDC domains, some members possess a 3′→5′ exonuclease domain in their N terminus (WRN and *Xenopus laevis* FFA-1), a nuclear localization signal in their C terminus (BLM and WRN), or acidic patches (BLM, WRN, RECQ1, RECQ4, Rqh1, or Sgs1) (4, 78, 149, 162). Recently, a new functionally conserved domain mediating strand annealing and strand exchange has been uncovered in the N terminus of Sgs1 and BLM (32) (**Figure 1**).

The quaternary structure of several RecQ helicases has been investigated. BLM forms principally hexameric ring structures, but a fourfold symmetric square form is also detected, which may represent a distinct oligomeric species or a side view of the hexameric form (76). Unlike BLM, active *E. coli* RecQ exists as a monomeric protein (164, 170). On the other hand, RECQ1 can form monomers or dimers (109, 117). Therefore, RecQ enzymes may adopt different structures (reviewed in 149).

An evolutionarily conserved feature of RecQ helicases is their physical association with the Top3 type IA topoisomerase (**Figure 2**). In budding and fission yeast, Sgs1 and Rqh1, respectively, interact physically with Top3 (1, 8, 46, 50). Of the two isoforms of TOP3 in humans, BLM interacts with TOP3α (73, 161), and RECQ5 interacts with either

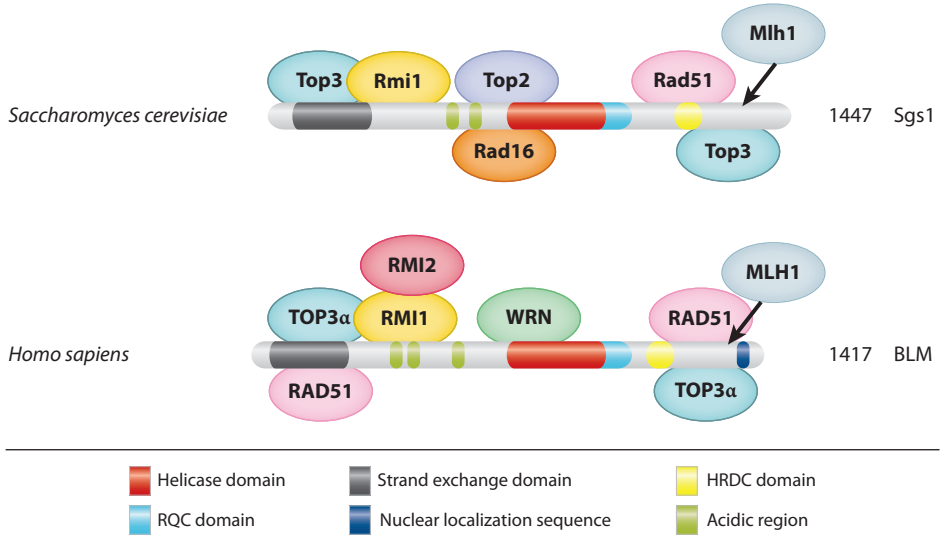

Figure 2

Conserved interactions between DNA repair and recombination proteins with Sgs1 and BLM. In yeast, Sgs1 physically interacts with many different repair and recombination proteins, such as Top3, Rmi1, Top2, Rad16, Mlh1, and Rad51, shown here as ovals. Many of these interactions are evolutionarily conserved in the human BLM protein. BLM also interacts with WRN, another RecQ helicase, and RMI1 interacts with RMI2. Arrows show the positions of residues critical for the interactions of each protein with Mlh1/MLH1. Although Sgs1 and BLM interact with other partners, only those DNA repair/replication proteins whose interaction regions are known are displayed. Colors for Sgs1 and BLM are as in **Figure 1**.

TOP3α or TOP3β (135). Similar interactions for the other human RecQ homologs have either not been tested or not been found. Recently, a second conserved partner was identified in yeast (Rmi1/Nce4) (29, 108) and human cells (RMI1/BLAP75) (168) (**Figure 2**). RMI1 proteins contain an OB-fold found in many DNA-binding proteins like RPA or Cdc13, a telomere-associated, ssDNA-binding protein (142), and biochemical studies have shown that Rmi1 forms a stable heteromeric complex with Sgs1-Top3 (BLM-TOP3α). Genetically, *rmi1* mutants behave like *top3* mutants (50, 151) and exhibit premature DNA-damage checkpoint activation (168). Rmi1 is a structure-specific DNA-binding protein with preferences for cruciforms that may help target Sgs1-Top3 to appropriate substrates (108). Rmi1-Top3 biochemical activity is independent of Sgs1, where Rmi1 stimulates Top3 by promoting its interaction with ssDNA and its superhelical relaxation activity (31). Recently, in mammalian cells, an additional partner, RMI2 (BLAP18), was found to interact with RMI1 through two OB-fold domains. RMI2, an integral component of the BLM complex, is important for the stability, localization, and function of the BLM complex in vivo (138, 163). It has been hypothesized that RMI multi-OB-folds mediate two modes of BLM action via RPA-mediated protein-DNA interaction, which promotes unwinding, and via RMI-mediated protein-protein interactions, which promotes dissolution.

In addition to Top3 and the Rmi subunits, RecQ helicases interact with many proteins involved in DNA replication and repair, although the exact nature of these interactions is poorly understood. Sgsl interacts with the type II topoisomerase Top2 (156), with Rad16/Pso5, a protein responsible for repair of silent DNA (128), with the Rad51 recombinase (160), and with the mismatch proteins Mlh3 (152) and Mlh1 (2, 41), as well as with Srs2 helicase and Mre11 nuclease, both of which can form various subcomplexes with Sgs1 (34) (**Figure 2**). On the mammalian side, BLM and WRN interact with each other (150), with RPA (18, 19, 134), and p53 (13, 139, 153). In addition, BLM interacts with the largest subunit of chromatin assembly factor 1 (CAF-1) (72) and, like Sgs1, also interacts with RAD51 (160) and MLH1 (41, 85) (**Figure 2**). BLM also has additional protein interactions not listed here.

Recently, another intriguing conserved interaction was discovered between Gis1, a yeast jumonji domain protein and both Sgs1 and the WRN protein (143). In yeast, Gis1 is a DNA damage–responsive effector capable of activating genes under conditions of stress (116, 143). Because the jumonji domain contains histone demethylase activity (144, 166), it raises the question of a connection between DNA repair and histone demethylation. The yeast Gis1 protein also interacts with the Nfi1/Siz2 SUMO ligase to form a complex. It is interesting that in mammals, several related jumonji-interacting proteins are found in promyelocytic leukemia nuclear bodies (PML NBs), which also contain transcription factors, BLM, WRN, and a SUMO ligase (13, 153, 171). Perhaps the jumonji-interacting proteins in yeast are acting in a fashion similar to that of PML NBs, where the recruitment of transcription factors modulates their activity in response to specific signals. However, it is unknown whether the activities of the RecQ helicases themselves are modulated in these bodies by posttranslational modification.

Genome instability: the aberrant rearrangement of chromosomal material that is often a prerequisite to cancer and aging

DOUBLE-STRAND BREAK REPAIR BY HOMOLOGOUS RECOMBINATION

Many types of DNA damage can lead directly or indirectly to the formation of DSBs. When DNA repair is misregulated, mutations and genomic rearrangements can occur that are precursors to tumorigenesis and even cellular death. One mechanism to repair DSBs is HR, which uses a homologous template for repair and is generally considered an error-free mechanism. In the absence of RecQ helicase function, genome instability is increased, consistent with an important function for these enzymes in HR. The RecQ proteins function at multiple

repair steps in HR, and therefore this repair pathway is the main focus of this review.

When a DSB occurs, the DNA ends are first recognized by the Mre11-Rad50-Xrs2 complex in budding yeast or the MRE11-RAD50-NBS1 complex in fission yeast and multicellular eukaryotes (DSB repair is reviewed in 94). In budding yeast, the DNA ends are then resected in a 5′ to 3′ direction by the endo/exonuclease activity of Mre11 in conjunction with the Sae2 endonuclease (89), revealing 3′ ssDNA overhangs. These initial processing steps are critical because they target the DNA for repair using a homologous template and inhibit other repair mechanisms such as nonhomologous end joining. The DNA ends are then further resected by one of two pathways, one that utilizes Exo1 and the other that uses Sgs1 and Dna2. The surprising involvement of the RecQ proteins in this processing step will be discussed in depth below. The ssDNA is subsequently coated by RPA, the ssDNA-binding protein. Rad52 then recruits Rad51 to the RPA-coated ssDNA, displacing RPA and leading to the formation of a Rad51 nucleoprotein filament. This Rad51-ssDNA filament performs a homology search followed by strand invasion leading to displacement loop (D-loop) formation. Several RecQ helicases have also been shown to function in disruption of Rad51 nucleoprotein filaments or by preventing D-loop formation. After formation of the D-loop, branch migration occurs. This structure can be resolved by second end capture of the homologous chromosome by two different mechanisms: (*a*) a process called synthesis-dependent strand annealing (SDSA), in which the DNA strand reanneals to the original template or (*b*) by formation of double Holliday junctions (dHJs) that can be resolved by the Sgs1-Top3-Rmi1/BLM-TOP3-RMI1 (BLAP75)-RMI2 (BLAP18) complex. Therefore, the RecQ proteins perform distinct functions during HR: aiding in the resection of the DSB, inhibiting Rad51 filaments and D-loop formation, and resolving dHJs. Some of these steps are also conserved in meiosis (114).

Displacement loop (D-loop): the single-stranded DNA formed when two strands of dsDNA are separated by the invasion of a third strand that anneals by base-pair complementation

Synthesis-dependent strand annealing (SDSA): a recombination process that occurs when an extended strand is displaced and base paired with a complementary single strand to create a duplex without a crossover

Holliday junction (HJ): cross-strand exchange between two DNA molecules that results in a four-way junction

Double Holliday junction (dHJ): two adjacent Holliday junctions formed between four strands of DNA

Resection: degradation of one of the complementary strands of DNA

ROLE OF YEAST AND HUMAN HELICASES IN DNA END RESECTION

After a DSB forms, DNA end resection leads to the formation of 3′ ssDNA tails (157). Although many of the key players involved in DNA end resection were known, it was hypothesized that there may be additional proteins involved. For example, in *E. coli*, the RecBCD complex, comprised of two helicases and a nuclease, is sufficient for generating 3′ ssDNA tails. However, in eukaryotes there is no corresponding helicase known to function in resection. Further analysis revealed that the Sgs1 complex in yeast and one of its human homologs, BLM, are also involved in the extensive resection that occurs after MRX and Sae2 protein function (60, 102, 111, 172). The involvement of Sgs1 and BLM in this process was unexpected because BLM has a well-established later role in resolution of dHJs (162).

One challenge in identifying additional proteins involved in DNA end resection is that the ssDNA signal is normally transient, being lost as breaks are repaired by homologous recombination. To circumvent this problem, repair by gene conversion can be blocked at an inducible DSB site by either disrupting *RAD51* or by eliminating donor sequences needed for strand invasion to occur. Using these approaches, two independent groups found that Sgs1 functions in DNA resection because *sgs1*Δ cells have a significantly slower rate of ssDNA formation (102, 172). In addition to the helicase activity of Sgs1, the Sgs1-interacting partners Rmi1 and Top3 are also necessary for end resection, suggesting that the entire Sgs1 complex is involved in this step (102, 172).

A breakthrough in our understanding of the resection pathway occurred when it was discovered that *sgs1*Δ *exo1*Δ double mutants showed substantially decreased DNA resection activity compared to the single *sgs1*Δ or *exo1*Δ strains (102, 172). These results suggest that Sgs1 and Exo1 function in different pathways that each contribute to end resection. Because RecBCD is the main pathway for end resection in

bacteria, it was hypothesized that the Sgs1 helicase may also function with a corresponding nuclease during resection. Further analysis revealed that this nuclease is Dna2 (172), which also functions in Okazaki fragment processing (5).

Interestingly, when a DSB is induced in cells in which both Sgs1 and Exo1 resection pathways are blocked, DNA fragments accumulate that are 50–100 nucleotides shorter than the initial cleaved fragment (102, 172). Subsequent analysis of these ssDNA intermediates revealed that their formation is dependent upon the MRX complex and Sae2 (102, 172). These observations lead to a two-step model for DNA end resection in which the ends are first resected by the MRX complex and Sae2 and subsequently resected by either the Sgs1-Dna2 or Exo1 pathway (**Figure 3**). The combined resection activities of these proteins ensure that sufficient ssDNA is generated for later steps in recombination.

Recently, resection has been reconstituted in vitro using purified yeast MRX, Sgs1, Rmi1, Top3, Dna2, and RPA proteins (24, 112). Similar to the genetic model proposed, Sgs1 helicase activity is needed to separate the DSB ends to provide access for nuclease cleavage. This reaction, which is mediated by RPA, has a strong specificity to remove nucleotides from the 5′ end. RPA also promotes DNA unwinding and enhances the 5′ endonuclease activity of Dna2 (112). In vitro, the Sgs1-Dna2-RPA protein complex is the minimal unit capable of resection, and its activity is stimulated by the addition of MRX and/or Rmi1-Top3 (24, 112). Sgs1 and Dna2 physically interact, even in the absence of DNA, suggesting that unwinding and resection of dsDNA must be concerted (24, 112). In contrast to the in vivo studies, which show that Sae2 is important during the initial steps of resection, in vitro its activity is dispensable (112). Furthermore, the topoisomerase activity of Top3, which is needed for crossovers, is dispensable for its function in resection (112). Further studies of the molecular details of these reactions will lead to deeper insights into the function of the RecQ helicases during resection.

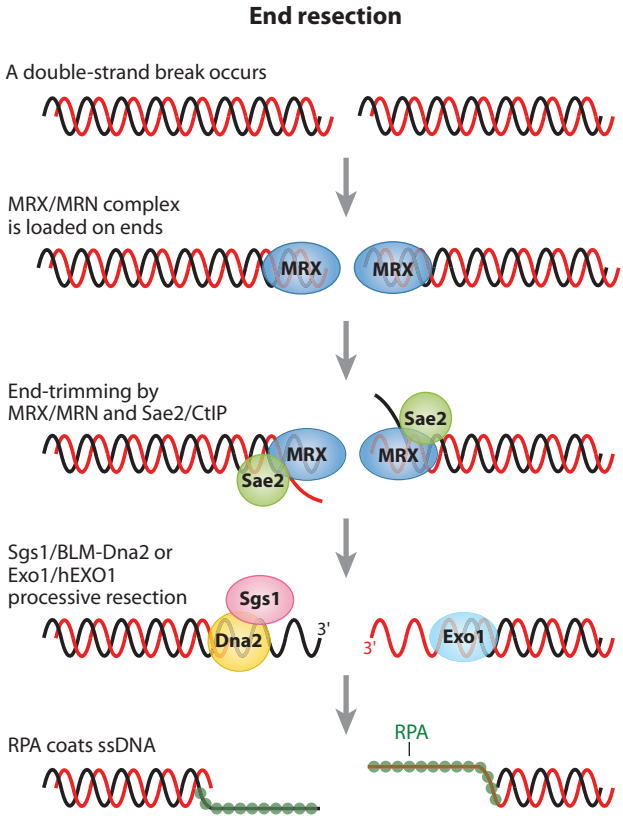

Figure 3

Model for RecQ function during end resection. After a double-strand break (DSB) occurs, the DNA ends are recognized by Mre11-Rad50-Xrs2 (MRX) in yeast or MRE11-RAD50-NBS1 (MRN) in mammals. The DNA ends are partially resected by Mre11/MRE11 nucleases, in collaboration with Sae2/CtIP endonucleases, leaving short 3′ single-stranded DNA tails. These DNA ends can be further resected by utilizing Sgs1/BLM helicases and Dna2/DNA2 nucleases (*left*) or via a parallel pathway that uses Exo1/EXO1 nucleases (*right*). The ssDNA created is coated by RPA.

Inhibition of DNA end resection has profound consequences on the DNA damage response. For example, blocking both end-resection pathways leads to increased susceptibility to DNA damaging agents, increased gross chromosomal rearrangements, and the inability to activate the DNA damage checkpoint under DNA damaging conditions (60). Importantly, these defects are not specific to yeast but are also observed in human cells. For example, cells simultaneously depleted for BLM and EXO1 have less activated CHK1, a

G-quadruplex: four-stranded structures formed by Hoogsteen base pairing of G-rich sequences

DNA-damage checkpoint protein, decreased RPA2 phosphorylation, and decreased survival rates after exposure to camptothecin, a DNA damaging agent (60). In addition, colocalization of H2AX and RPA foci markers of DSBs and ssDNA, respectively, is decreased in these cells, suggesting that less ssDNA is created when these two pathways are blocked in mammalian cells, which is similar to that observed in yeast (60).

Purified human BLM and EXO1 have also been assayed in vitro for resection of synthetic DNA substrates (111). Unlike the in vivo observations for yeast and human cells, purified BLM and EXO1 act together in vitro to resect 5′ to 3′ dsDNA templates even in the absence of DNA2 (111). The resection activity of BLM and EXO1 promotes RAD51 joint molecule formation. Importantly, the resection activity is specific to BLM because no other RecQ homolog stimulates resection (111). Interestingly, in *X. laevis* extracts, the WRN protein and DNA2 were similarly found to function in resection of DSB ends (84). It remains an open question as to why the in vivo analysis suggesting that Sgs1/BLM function independently of Exo1/EXO1 and the in vitro analysis suggesting that BLM stimulates EXO1 activity do not correspond.

ROLE OF RECQ HOMOLOGS IN THE RECOGNITION AND RESOLUTION OF SECONDARY DNA STRUCTURES

Purification and characterization of RecQ proteins from bacteria, yeast, plants, and mammals have revealed that RecQ helicases unwind a wide variety of DNA substrates, with a marked preference for HJs, G-quadruplexes, and D-loops (7, 25, 61, 106, 140, 145). These helicases not only bind ssDNA but can also bind blunt four-stranded junction structures, indicating that they do not always require a free 3′ DNA tail (7, 25). Interestingly, although early work on a truncated purified version of Sgs1 indicated poor processivity of the helicase (9), recently an unprecedented strong helicase activity for the full-length Sgs1 protein (25), far beyond that of the BLM and WRN homologs, has been shown (19, 134). In addition, the full-length Sgs1 unwinds a wider variety of substrates with even greater efficiency than the truncated form, although it still has a preference for unwinding HJs (25).

An early task of recombinational repair is homology search and strand invasion, which can be divided into a few key steps (**Figure 4**). In one of the first steps, Rad51 coats the ssDNA and replaces RPA. Subsequently, Rad51 nucleoprotein filaments search for complementary sequences in dsDNA. During strand invasion and pairing, the noncomplementary strand of the duplex is displaced, creating a D-loop, a process that can occur at collapsed replication forks (**Figure 4**). In vitro, Rad51 efficiently forms D-loops with either a 5′ or a 3′ invaded end. However, only the 3′ invaded end is proficient for priming new DNA synthesis to allow extension of the D-loop by DNA polymerase. The cross-strand structures formed during this process can also branch migrate. In vitro studies indicate that the RecQ helicases may regulate many steps during strand invasion and exchange. For example, RECQ5 and BLM can disrupt the initial step of RAD51 filament formation (65, 21), similar to the Srs2 protein in yeast (82, 147). It has also been shown that several RecQ helicases can disrupt D-loops. For example, *Caenorhabditis elegans* WRN-1 and *Arabidopsis thaliana* RECQ2 unwind D-loops (68, 81). In vitro, mammalian BLM and RECQ1 preferentially melt naked D-loops and show an affinity for those with a 5′ invaded end. Therefore, these helicases selectively dissociate recombination intermediates whose polarity is unfavorable for polymerase extension (3, 145) (21). Interestingly, BLM disruption of D-loops is restricted to filaments containing an inactive ADP-bound form of RAD51, suggesting that the filament remains inactive and susceptible to BLM dissociation until the cell is fully prepared for HR. During the latter steps of the strand exchange reaction, human RECQ1 efficiently promotes 3′ → 5′ three-stranded branch migration of the D-loop (20).

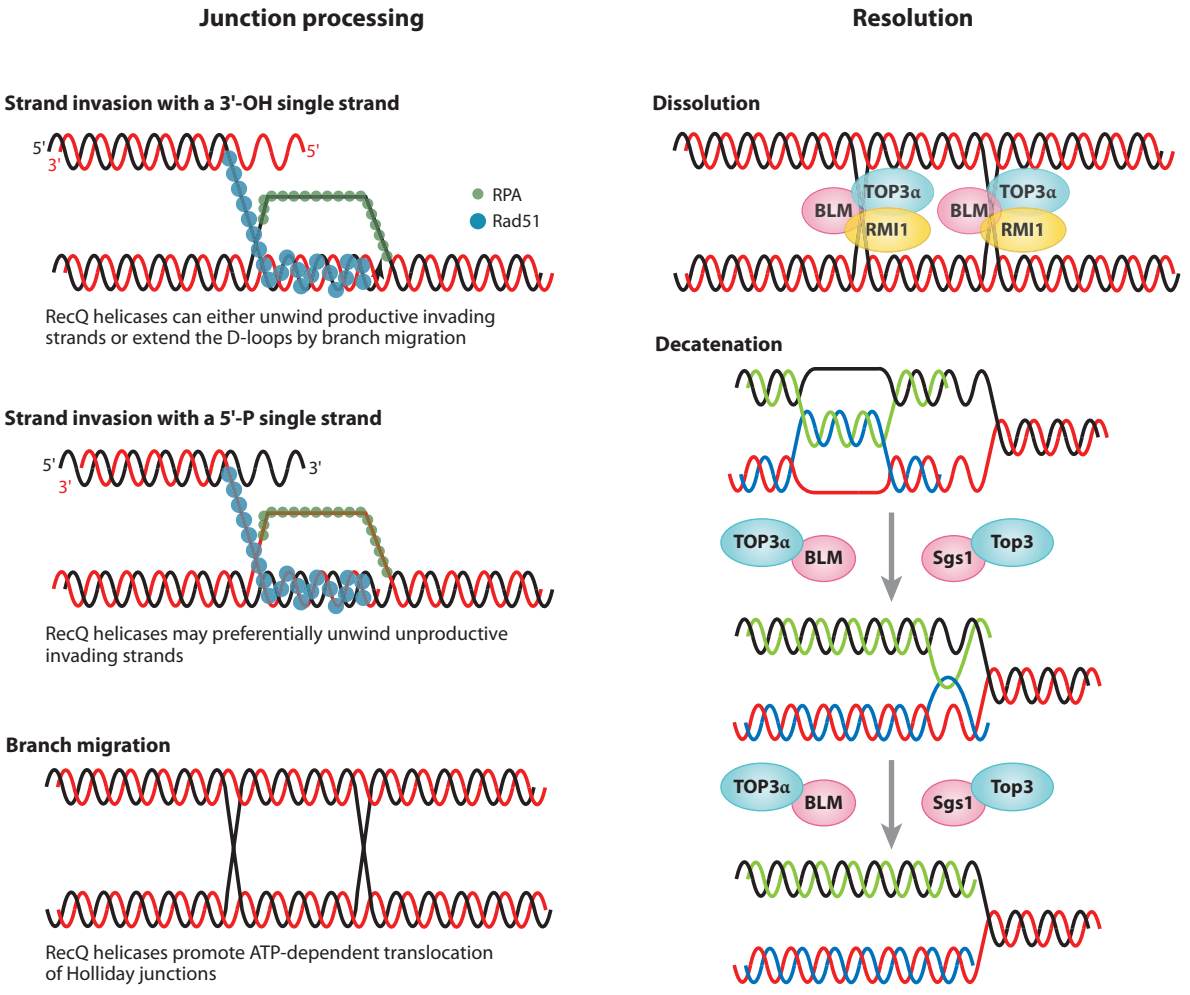

Figure 4

Model for RecQ function during junction processing and resolution. Junction processing (*left*): The Rad52 group of proteins recruits Rad51 and displaces RPA, leading to Rad51 filament formation. Rad51 filaments perform homology search and strand invasion, leading to displacement-loop (D-loop) formation followed by branch migration. Rad51-coated DNA can invade using 5′ or 3′ DNA ends; however, only the 3′ invading end can prime DNA synthesis for homologous recombination (HR). The RecQ proteins can unwind the 5′ end and thus abort the unproductive reaction. They can also unwind the 3′ end after it is extended, to promote synthesis-dependent strand annealing (*not shown*). Alternatively, second end capture of the 3′ tail of the other broken end can lead to double Holliday junction (dHJ) formation. The RecQ proteins function by promoting translocation and branch migration of dHJs. Resolution (*right*): Dissolution of dHJs utilizes the Sgs1-Top3-Rmi1/BLM-TOP3α-RMI1(BLAP75) complex. The mammalian proteins are shown bound to each Holliday junction (HJ), and if they move toward each other, dissolution can occur. During DNA replication, hemicatenanes are thought to form behind the replication fork (17). In vitro, maximal decatenation of this structure is achieved when RMI1 and RMI2 are added to the reaction.

During recombination, branch migration of the D-loop junction can occur, and subsequent processing of the HJ determines the outcome of the reaction. In prokaryotes, RecQ or RuvA/RuvB promote ATP-dependent branch migration of HJs. In fission yeast, replication fork arrest results in accumulation of HJs in the absence of Rqh1, which can either impede sister-chromatid segregation or lead to the formation of recombinants through HJ

resolution (42). In vitro, BLM and WRN promote the ATP-dependent translocation of HJs, and binding of recombinant p53 attenuates their ability to unwind synthetic HJs (167). RECQ1 predominantly branch migrates HJs in an ATP-dependent fashion in human nuclear extracts (90).

It has long been suspected that a dHJ could be resolved by a topoisomerase partnered with a helicase by convergent branch migration of the HJs (23, 55, 110). Genetic analysis of budding yeast Top3 and Sgs1 supports this hypothesis. Deletion of Sgs1 increases both spontaneous and DSB-induced crossovers, suggesting that Sgs1 with Top3 removes dHJ intermediates from a crossover-producing repair pathway (69, 124). In an elegant in vitro system, it was shown that BLM and TOP3α resolve recombination intermediates containing a dHJ through a mechanism called double-junction dissolution, a process that prevents exchange of flanking sequences (162). This mechanism is conserved in flies (118), but it is important to note that the in vitro systems only allow the readout of the dissolution reaction, whereas a dHJ could equally be branch migrated in either direction or apart from one another, leading to an increase in the distance between the two junctions. However, the dissolution reaction is highly specific for BLM and depends upon a functional HRDC domain (159). The discovery of BLAP75/RMI1 as a third member of the Sgs1-Top3 (BLM-TOP3α) complex led to the notion of a dissolvasome. Under physiological conditions, dHJ dissolution depends completely on RMI1 to limit DNA crossover formation (122). The conserved N-terminal third of RMI1 mediates complex formation with TOP3α and BLM and acts by recruiting TOP3α to dHJs. Hence, the activity of RMI1 is specific for dissolution catalyzed by TOP3α (22, 123, 158). Similar results have recently been shown for the yeast proteins (26). The newest member of this complex found in mammalian cells, RMI2, was shown to stimulate dHJ dissolution (138).

G-quadruplex DNAs, which can form into non-Watson-Crick structures within guanine-rich DNA sequences, are another DNA structure that can be preferentially unwound by RecQ helicases (**Figure 5**). These sequences are often found in telomeric or rDNA repeats. Most RecQ family DNA helicases are able to unwind G-quadruplex DNA (120). The efficient unwinding of G-G paired DNA by Sgs1 is ATP- and $Mg2^{+}$-dependent and requires a short 3′ single-stranded tail. Diminished ability to unwind G-G paired regions may explain the deleterious effect of mutations in Sgs1 on rDNA stability and the accelerated aging of yeast lacking Sgs1, as well as humans deficient in the WRN helicase (140). For the mammalian proteins, the helicase activities of BLM and WRN are needed for their G-quadruplex binding capabilities (92). BLM and Sgs1 preferentially unwind G4 DNA relative to HJs. This substrate preference reflects binding affinity and maps to the helicase domain. These observations suggest that, in addition to their roles in promoting recombination to restart a stalled fork, BLM and Sgs1 also function in DNA replication to remove G4 DNA structures, which could hinder fork progression (67).

MEIOTIC FUNCTIONS

There are several observations that point to a role for the RecQ family in meiosis. For example, in budding yeast, *sgs1*Δ mutants exhibit both reduced tetrad formation and spore viability (49, 156). Human females homozygous for BLM mutations display reduced fertility, and males are infertile (reviewed in 51). In addition, BLM protein detected by immunofluorescence frequently localizes to sites of recombination as discrete foci on synapsed cores with the recombinases RAD51 and DMC1, providing cytological evidence that BLM functions in meiotic recombination as well (105). Furthermore, evidence from other eukaryotes also supports the notion that RecQ family members are involved in meiosis. For example, mutations in the Bloom homolog in flies (*mus309*) and worms (*him-6*) are infertile (59, 63, 83).

In budding yeast, the decrease in spore viability in *sgs1* mutants was attributed to meiosis I missegregation (156) and precocious separation

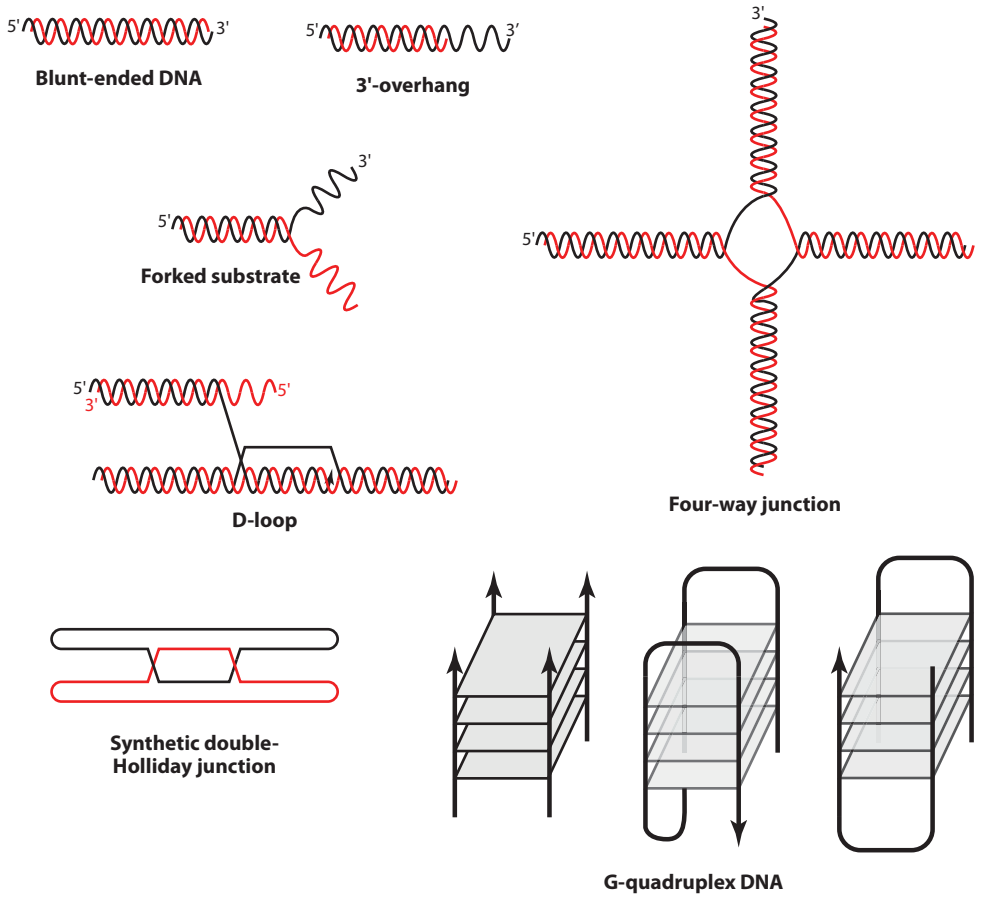

Figure 5

DNA structures unwound by one or more RecQ helicases. The RecQ proteins unwind a diverse set of DNA structures in vitro, such as blunt-ended DNA (however, not the preferred substrate), 3′overhangs, forked substrates (such as those that arise during DNA replication), D-loops that arise during strand invasion, four-way junctions [similar to a Holliday junction (HJ)], synthetic double Holliday junctions (dHJs; mimicking a recombination intermediate), or structures capable of forming G-quadruplex DNA (e.g., predicted in rDNA and telomere sequences).

of sister chromatids (126), whereas heteroallelic recombination and crossovers are modestly elevated (155). Further studies have indicated that, in the absence of Sgs1, there is a long delay in the appearance of four-spored asci dependent on the initiation of recombination (49). Indeed, disruption of *RED1* or *RAD17*, two checkpoint genes, partially alleviates the poor sporulation of *sgs1* mutants. Interestingly, only the region of Sgs1 spanning amino acids 126 to 596 is required for the meiotic function—the helicase function per se is dispensable (104). However, another study suggests that helicase activity is required in meiosis (125), perhaps reflecting complex interactions between the domains of Sgs1 (97). An important breakthrough in understanding Sgs1 meiotic function came from the discovery that the absence of Sgs1 leads to an increase in both the number of interhomolog connections and the number of crossovers without affecting the frequency of noncrossover events (125). These observations assign a new role for Sgs1 in the negative regulation of meiotic crossovers at an early stage of

meiotic prophase. A similar function was later found for both spontaneous and DSB-induced mitotic events (69, 124). In subsequent meiotic studies, the *sgs1*-ΔC795 allele, which truncates the protein after amino acid 795, has been used instead of the complete deletion. Although the *sgs1*-ΔC795 mutants are indistinguishable from the null mutant with respect to the synapsis and crossover phenotypes, they sporulate like wild-type cells, exhibit significantly improved spore viability compared to *sgs1*Δ (125), and grow as well as wild-type cells (107). One explanation is that mitotic chromosome instability in *sgs1*Δ diploids leads to elevated levels of aneuploidy, which may result in poor sporulation and spore viability. It is also possible that some meiotic functions of Sgs1 are mediated via interactions with other partners like Top2 or Top3, whose interacting sites are still present in the *sgs1*-ΔC795 mutant.

The molecular mechanisms by which Sgs1 regulates meiotic crossovers was further revealed when it was found that the SIC/ZMM proteins, responsible for homologous chromosomes' synapsis and crossovers, antagonize the anticrossover activity of Sgs1 (71, 113). Elegant genetic and molecular work reached the conclusion that Sgs1 and Mus81/Mms4 can disrupt the formation of joint molecules comprised of three and four interconnected duplexes that result from secondary strand-invasion events, therefore enhancing the efficiency of repair (70, 113, 114). These results indicate that, in budding yeast meiosis, Sgs1 acts as a dissolvase (118, 162) and helps disrupt D-loop structures (3, 7, 115). Interestingly, the role of Sgs1 in meiosis is not fully conserved. Compelling data from fission yeast indicate that, unlike Sgs1, Rqh1 promotes meiotic recombination (39). This difference between RecQ meiotic function in *S. cerevisiae* and *Schizosaccharomyces pombe* could be related to the absence of the synaptonemal complex and the lack of crossover interference in fission yeast. However, it cannot be ruled out that *S. pombe* Rqh1, unlike Sgs1, plays a role in meiotic DSB resection or that the requirement for Rqh1 in interhomolog recombination is more pronounced.

REPAIR OF REPLICATIVE DAMAGE

Many of the activities performed by the RecQ helicases are also important at multiple steps during DNA replication. The RecQ helicases help enable the association of the polymerases with the replication fork, unwind DNA structures that potentially lead to replication fork stalling (i.e., G-quadruplexes and hairpin structures), and resolve hemicatenane-like structures that can form during repair of replicative damage. In the absence of RecQ activity, errors lead to stalled or collapsed replication forks, which need to be repaired to maintain genomic integrity and to prevent DSBs. RecQ proteins may help unwind inappropriately paired nucleic acids during replication to prevent inappropriate HR events and therefore maintain genomic stability.

One of the first lines of evidence suggesting that the RecQ proteins can function in the repair of replicative damage comes from the observation in yeast that expression of the *SGS1* gene is cell-cycle regulated and peaks in S phase (45). Similarly, BLM and RECQ4 are cell-cycle regulated, and their concentration peaks during S phase (43, 54, 165), whereas the other mammalian RecQ homologs show no such regulation. RecQ proteins also interact with many DNA replication proteins. For example, in yeast, Sgs1 physically interacts with topoisomerases I and II and RPA (36, 50, 156). Sgs1 is also found in chromatin immunoprecipitation (ChIP) experiments at unperturbed replication forks (36). Similarly, the human homolog WRN interacts with RPA, PCNA, POLδ, and TOPO1 and copurifies with the DNA replication complex in ES cells (18, 74, 87, 88). Indeed, upon hydroxyurea treatment, WRN localizes to discrete nuclear foci that coincide with those formed by RPA, suggesting that WRN prevents aberrant recombination events at sites of stalled replication forks by dissociating recombination intermediates (37, 75). The BLM proteins interact with p53 and RAD51 at stalled replication forks (132). BLM also forms foci that colocalize with PCNA at stalled replication forks and with the BRCA1-associated

genome surveillance (BASC) complex (154). Finally, RECQ4 is an integral component of the MCM complex, where MCM10 is important for the RECQ4 interaction with the other replisome components, MCM2–7 (165).

What role(s) does the RecQ helicases play at the replication fork? Perhaps the topological constraints of DNA replication generate catenane-like structures that require DNA helicases and topoisomerases for resolution. In this scenario, the RecQ proteins act on DNA structures enabling replication to continue efficiently (148). Without the concerted action of the RecQ helicases with DNA topoisomerases, aberrant replication structures arise. In fact, disruption of RecQ helicases leads to the accumulation of abnormal replication intermediates in both BLM- and WRN-deficient cells (96, 119). Surprisingly, in budding yeast, *sgs1*Δ cells progress through S phase faster than wild-type cells (148). However, not all genomic loci replicate faster because the rDNA actually replicates more slowly in *sgs1*Δ (148). Furthermore, disruption of *SGS1* causes contractions at trinucleotide repeats (77). Both rDNA and some trinucleotide repeat sequences (such as CTG repeats) are capable of forming G-quadruplexes, suggesting that such sequences in these repetitive units require RecQ helicase activity to replicate efficiently. In addition, the reliance on other repair mechanisms, such as sister-chromatid recombination or single-strand annealing, in the absence of RecQ helicases may lead to slower processivity during the replication of highly repetitive elements.

The RecQ helicases also function in the repair of replication-induced damage (**Figure 4**). Our understanding of the RecQ proteins in DNA replication comes mostly from experiments that utilize drugs that stall or collapse replication forks. For example, hydroxyurea (HU) is frequently used to deplete dNTP pools and therefore initially stall and then subsequently collapse forks. Methyl methanesulfonate (MMS) alkylates DNA and also indirectly leads to replication fork stalling. Using these drugs to perturb replication, Sgs1 was shown to contribute to the polymerases' ability to efficiently immunoprecipitate with the replication fork (36). When replication fork damage is induced with MMS, X-shaped molecules form at replication origins, which are revealed by two-dimensional gel electrophoresis. These X-structures likely contain ssDNA because their formation is sensitive to Mung Bean nuclease (93). Complete disruption of *SGS1* or a point mutation in its helicase domain leads to the accumulation of these X structures at damaged forks (10, 93). Furthermore, X-structure accumulation in *sgs1*Δ cells is dependent upon the recombination protein Rad51 and a postreplication repair protein Rad18, suggesting that a template-switching mechanism is important for their formation (17, 93). The Rad18-mediated postreplicative repair pathway requires the SUMO-conjugating enzyme Ubc9 and sumoylated PCNA, suggesting that assembly/disassembly of proteins at the site of replicative damage is an important contributor to X-structure formation (17). Sgs1 itself is also a Ubc9-dependent SUMO target (16). Interestingly, it is possible to isolate mutants of *SGS1* that encode proteins defective in repair of replicative damage but do not influence recombination at other loci, indicating that the function of Sgs1 in repair of replicative damage is distinct from its function in resolution of HR intermediates (10).

X-structures: replication intermediates that form in the presence of DNA-damaging agents that contain stretches of ssDNA

When recombination or replication intermediates are not properly resolved during S phase, cells are unable to undergo sister-chromatid disjunction. In this scenario, anaphase bridges (or ultrafine bridges), representing incomplete chromosome segregation, connect the two nuclei. Interestingly, BLM, along with TOP3α and RMI1, localize to these bridges and are needed for their resolution (27). The observed centromeric anaphase bridges also contain the PICH helicase (6), where PICH is first recruited to the bridges followed by BLM, which then resolves them (27). Intriguingly, FANCD2/I proteins, which are ubiquitylated and localize to DNA damage sites, are found at the termini of a subset of BLM-dependent ultrafine bridges formed at fragile sites (28).

Disruption of RecQ function during replicative repair also activates the DNA damage checkpoint. The intra-S phase checkpoint is largely mediated by the Rad53 kinase in yeast and the corresponding CHK2 kinase in metazoans. Rad53 is activated by DNA damage and becomes phosphorylated, leading to a signaling cascade that transduces the damage signal. Sgs1 is important for Rad53 activation (12). Sgs1 directly binds Rad53, suggesting that they interact at damaged DNA structures for checkpoint activation (12, 45). Sgs1 and Rad53 also form foci that colocalize with the replication factor Orc2 (45). In mammalian cells, ATR, a kinase upstream to CHK2, binds to and phosphorylates BLM (40). Furthermore, when replication forks are stalled with HU, BLM foci colocalize with another kinase, CHK1, and 53BP1, a p53-interacting protein that participates in the DNA damage response (133). When *CHK1* mRNA is depleted, the formation of BLM and 53BP1 foci at replication forks is disrupted, suggesting that CHK1 recruits BLM to sites of replicative damage (133). In summary, both yeast and mammalian RecQ proteins are important mediators of the DNA damage checkpoint in response to replication fork stalling and collapse.

TELOMERES

Maintenance of telomere sequences at chromosome ends is critical for genomic stability. Telomeres consist of G-rich repetitive DNA sequences. In the absence of telomerase, telomeres shorten after each cell division until the cell senesces. To bypass senescence, telomeres can be elongated by different mechanisms; for example, one that utilizes telomerase, an RNA-protein complex, or secondly by alternative lengthening of telomeres (ALT), which is predominately DNA recombination mediated. In budding yeast, lengthening of telomeres by ALT requires the recombination protein Rad52. In human tumors, cells often evade senescence by activating either pathway to extend telomere length. The RecQ proteins have been implicated to function in telomere maintenance in the ALT pathway (11). The WRN RecQ helicase has a well-established role at telomeres in humans. WRN is important for lagging strand synthesis and for efficient replication of G-rich telomeric DNA (38). As mentioned previously, the WRN mutant mouse only recapitulates Werner syndrome when combined with telomerase mutant alleles, suggesting that loss of WRN function at telomeres is critical in the development of this syndrome.

Telomere ends have 3′ overhangs, similar to those seen at processed DSBs, due to the protrusion of the G strand over the complementary C strand. In yeast, the length of the single-stranded G tails is approximately 12–14 nucleotides; G tails form in mice and humans as well and are considerably longer 75–300 nucleotides (86, 141). Studies in yeast have shown that formation of the G-tails is partially dependent on the MRX complex (86). Disruption of this complex does not completely abolish G-tail formation, suggesting that other proteins are also involved in the 5′ to 3′ processing of telomere ends (86).

One of the defining features of telomeres is their G-rich nature, potentially enabling the formation of G-quadruplexes, which are guanine-guanine interactions stabilized by interstrand pairings between four DNA strands. As discussed above, several RecQ proteins unwind G-quadruplex DNA in vitro including BLM, WRN, and Sgs1 (92, 140). In addition, like its role at DSBs (60, 102, 111, 172), Sgs1 is important in resection at telomeres. In budding yeast, an inducible cut site was used to generate a DSB adjacent to the left telomere of chromosome VII to monitor a single telomere for formation of ssDNA and lengthening (15). After induction of the DSB, the fragment distal to the break is lost and the short telomeric seed sequence is elongated. Wild-type cells can elongate this telomere seed sequence, but cells that are unable to form 3′ tails do not. Interestingly, using this assay, all the proteins required for resection of DSBs are also necessary for telomeric C-strand degradation and telomere elongation (i.e., Sae2, MRX, Sgs1, Exo1, and Dna2) (15). Similar to what occurs

at DSBs, Sgs1 and Dna2 function together in a pathway that is parallel to Exo1 in the resection of telomeric DNA (15). Additionally, the wild-type chromosomes observed in multiple mutants blocking both pathways (i.e., *exo1*Δ *sgs1*Δ or *sae2*Δ *exo1*Δ) did not exhibit any changes in their telomere lengths even though the cells were largely defective in extending the DSB-induced short telomere (15). Together, these results indicate that, similar to DSBs at other sites, resection of telomere ends is also partially mediated by Sgs1.

Interestingly, when resection is blocked at a site-specific DSB in an *sgs1*Δ *exo1*Δ double mutant strain, de novo telomere formation occurs at nontelomeric sequences (35, 100). At this site, telomere formation requires a telomeric seed sequence (short 1-5 GT-rich sequences) (35) and telomerase (100). Thus, Sgs1 and Exo1 normally inhibit telomere formation at nontelomeric DNA ends. Importantly, Pif1, a protein that directly interacts with telomerase, similarly inhibits de novo telomere formation at nontelomeric DSBs, albeit through a different mechanism (35). These observations are consistent with the idea that competition at a DSB end favors Cdc13-Stn1-Ten1, a telomerase specific RPA-like DNA binding protein complex, over RPA when GT sequences are short, which in turn leads to telomere addition (35). However, when Sgs1 and Exo1 are active, the longer single-stranded region that is formed enables more RPA to bind, thus preventing telomere addition at nontelomeric ends. Consistent with this view, blocking resection at a site-specific DSB in an *sgs1*Δ *exo1*Δ *sae2*Δ triple mutant eliminates de novo telomere addition (100).

Finally, it was recently shown that sumoylation of Sgs1 (98) in budding yeast or Rqh1 in fission yeast (127) is important for telomere-telomere recombination. Importantly, Sgs1 sumoylation did not influence recombination at other genomic loci, suggesting that the role of Sgs1 at telomeres may be distinct from its role at other loci (98).

Sumoylation: posttranslational covalent attachment of a small ubiquitin-like molecule (SUMO)

CONCLUDING REMARKS

The RecQ helicases have a diverse role in genome maintenance that, when unregulated in humans, can lead to premature aging and tumorigenesis. Although the diseases associated with mutations in the RecQ helicases are distinct, they are fundamentally characterized by increased cancer incidence, which is likely attributable to RecQ function in DNA repair. Recently, RecQ proteins were shown to function in resection of DSBs. This resection activity is also important for the ALT pathway, which is used during telomere extension in the absence of telomerase. However, it is unknown whether this function is important during the repair of replicative damage or during meiosis. In meiosis, it is likely that the RecQ role in dissolution is the predominant function. The RecQ proteins, in vitro, unwind a diverse set of DNA substrates, suggesting that it will be a continuing challenge to dissect out their specific functions at the DNA structures that it encounters. Furthermore, the role of posttranslational modification of RecQ, such as sumoylation, may be critical in the regulation of this important helicase family. Finally, insight into the precise biochemical function of this helicase family will be revealed by analyzing the specific structures that form from RecQ dysfunction, such as X structures that arise during DNA replication or the anaphase bridges found during mitosis.

FUTURE ISSUES

1. Is there a role for the RecQ proteins in end resection during meiosis or during DNA replication?

2. Are the phenotypes observed in human patients with RecQ disorders and their clinical symptoms attributed to the different functions of the RecQ protein at different processing steps?
3. What is the DNA-protein structure of the anaphase bridges, and do they occur in other eukaryotes?
4. Is the role of sumoylation of RecQ proteins only important at telomeres, or is it also important for its regulation in general, such as during DNA replication or recombination?
5. How are RecQ proteins recruited during repair of different DNA structures? How do they function in the processing of DNA lesions?
6. What is the specificity of the RecQ helicases in organisms with multiple RecQ proteins?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Michael Chang, Neil Hunter, Sarah Lambert, Lorraine Symington, and Peter Thorpe for helpful comments and careful reading of the manuscript. We thank Patrick Sung and Stephen Kowalczykowski for communicating unpublished results. This study was supported by ANR-07-BLAN-0350-01 (SG) and by National Institute Health grants GM50237 (RR), GM078840 (KAB), GM088413 (KAB).

LITERATURE CITED

1. Ahmad F, Stewart E. 2005. The N-terminal region of the *Schizosaccharomyces pombe* recQ helicase, Rqh1p, physically interacts with topoisomerase III and is required for Rqh1p function. *Mol. Genet. Genomics* 273:102–14
2. Argueso J, Kijas A, Sarin S, Heck J, Waase M, Alani E. 2003. Systematic mutagenesis of the *Saccharomyces cerevisiae* MLH1 gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. *Mol. Cell. Biol.* 23:873–86
3. Bachrati C, Borts R, Hickson I. 2006. Mobile D-loops are a preferred substrate for the Bloom's syndrome helicase. *Nucleic Acids Res.* 34:2269–79
4. Bachrati C, Hickson I. 2003. RecQ helicases: suppressors of tumorigenesis and premature aging. *Biochem. J.* 374:577–606
5. Bae SH, Bae KH, Kim JA, Seo YS. 2001. RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. *Nature* 412:456–61
6. Baumann C, Korner R, Hofmann K, Nigg EA. 2007. PICH, a centromere-associated SNF2 family ATPase, is regulated by Plk1 and required for the spindle checkpoint. *Cell* 128:101–14
7. Bennett RJ, Keck J, Wang J. 1999. Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of *S. cerevisiae*. *J. Mol. Biol.* 289:235–48
8. Bennett RJ, Noirot-Gros M, Wang J. 2000. Interaction between yeast Sgs1 helicase and DNA topoisomerase III. *J. Biol. Chem.* 275:26898–905
9. Bennett RJ, Sharp JA, Wang JC. 1998. Purification and characterization of the Sgs1 DNA helicase activity of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273:9644–50

10. Bernstein KA, Shor E, Sunjevaric I, Fumasoni M, Burgess RC, et al. 2009. Sgs1 function in the repair of DNA replication intermediates is separable from its role in homologous recombinational repair. *EMBO J.* 28:915–25
11. Bhattacharyya S, Sandy A, Groden J. 2010. Unwinding protein complexes in ALTernative telomere maintenance. *J. Cell Biochem.* 109:7–15
12. Bjergbaek L, Cobb JA, Tsai-Pflugfelder M, Gasser SM. 2005. Mechanistically distinct roles for Sgs1p in checkpoint activation and replication fork maintenance. *EMBO J.* 24:405–17
13. Blander G, Kipnis J, Leal JFM, Yu CE, Schellenberg GD, Oren M. 1999. Physical and functional interaction between p53 and the Werner's syndrome protein. *J. Biol. Chem.* 274:29463–69
14. Bloom D. 1954. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. *AMA Am. J. Dis. Child.* 88:754–58
15. Bonetti D, Martina M, Clerici M, Lucchini G, Longhese MP. 2009. Multiple pathways regulate 3′ overhang generation at *S. cerevisiae* telomeres. *Mol. Cell* 35:70–81
16. Branzei D, Sollier J, Liberi G, Zhao X, Maeda D, et al. 2006. Ubc9- and Mms21-mediated sumoylation counteracts recombinogenic events at damaged replication forks. *Cell* 127:509–22
17. Branzei D, Vanoli F, Foiani M. 2008. SUMOylation regulates Rad18-mediated template switch. *Nature* 456:915–20
18. Brosh RM Jr, Orren DK, Nehlin JO, Ravn PH, Kenny MK, et al. 1999. Functional and physical interaction between WRN helicase and human replication protein A. *J. Biol. Chem.* 274:18341–50
19. Brosh RM, Li JL, Kenny MK, Karow JK, Cooper MP, et al. 2000. Replication protein A physically interacts with the Bloom's syndrome protein and stimulates its helicase activity. *J. Biol. Chem.* 275:23500–8
20. Bugreev D, Brosh RJ, Mazin A. 2008. RECQ1 possesses DNA branch migration activity. *J. Biol. Chem.* 283:20231–42
21. Bugreev D, Yu X, Egelman E, Mazin A. 2007. Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. *Genes Dev.* 21:3085–94
22. Bussen W, Raynard S, Busygina V, Singh A, Sung P. 2007. Holliday junction processing activity of the BLM-Topo IIIalpha-BLAP75 complex. *J. Biol. Chem.* 282:31484–92
23. Caspari T, Murray JM, Carr AM. 2002. Cdc2-cyclin B kinase activity links Crb2 and Rqh1-topoisomerase III. *Genes Dev.* 16:1195–208
24. Cejka P, Cannavo E, Polaczek P, Masuda-Sasa T, Pokharel S, et al. 2010. DNA end resection by the Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature* In press
25. Cejka P, Kowalczykowski SC. 2010. The full-length *Saccharomyces cerevisiae* Sgs1 protein is a vigorous DNA helicase that preferentially unwinds Holliday junctions. *J. Biol. Chem.* 285:8290–301
26. Cejka P, Plank JL, Bachrati CZ, Hickson ID, Kowalczykowski SC. 2010. Rmi1 stimulates decatenation of double Holliday junctions during dissolution by Sgs1-Top3. *Nat. Struct. Mol. Biol.* In press
27. Chan KL, North PS, Hickson ID. 2007. BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges. *EMBO J.* 26:3397–409
28. Chan KL, Palmai-Pallag T, Ying S, Hickson ID. 2009. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nat. Cell Biol.* 11:753–60
29. Chang M, Bellaoui M, Zhang C, Desai R, Morozov P, et al. 2005. *RMI1/NCE4*, a suppressor of genome instability, encodes a member of the RecQ helicase/Topo III complex. *EMBO J.* 24:2024–33
30. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, et al. 2004. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat. Genet.* 36:877–82
31. Chen C, Brill S. 2007. Binding and activation of DNA topoisomerase III by the Rmi1 subunit. *J. Biol. Chem.* 282:28971–79
32. Chen CF, Brill SJ. 2010. An essential DNA strand-exchange activity is conserved in the divergent N-termini of BLM orthologs. *EMBO J.* 29:1713–25
33. Chester N, Kuo F, Kozak C, O'Hara CD, Leder P. 1998. Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. *Genes Dev.* 12:3382–93

34. Chiolo I, Carotenuto W, Maffioletti G, Petrini J, Foiani M, Liberi G. 2005. Srs2 and Sgs1 DNA helicases associate with Mre11 in different subcomplexes following checkpoint activation and CDK1-mediated Srs2 phosphorylation. *Mol. Cell. Biol.* 25:5738–51
35. Chung WH, Zhu Z, Papusha A, Malkova A, Ira G. 2010. Defective resection at DNA doubled-strand breaks leads to de novo telomere formation and enhances gene targeting. *PLoS Genet.* 6(5):e1000948
36. Cobb JA, Bjergbaek L, Shimada K, Frei C, Gasser SM. 2003. DNA polymerase stabilization at stalled replication forks requires Mec1 and the RecQ helicase Sgs1. *EMBO J.* 22:4325–36
37. Constantinou A, Tarsounas M, Karow JK, Brosh RM, Bohr VA, et al. 2000. Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep.* 1:80–84
38. Crabbe L, Verdun RE, Haggblom CI, Karlseder J. 2004. Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306:1951–53
39. Cromie G, Hyppa R, Smith G. 2008. The fission yeast BLM homolog Rqh1 promotes meiotic recombination. *Genetics* 179:1157–67
40. Davies SL, North PS, Dart A, Lakin ND, Hickson ID. 2004. Phosphorylation of the Bloom's syndrome helicase and its role in recovery from S-phase arrest. *Mol. Cell. Biol.* 24:1279–91
41. Dherin C, Gueneau E, Francin M, Nunez M, Miron S, et al. 2008. Characterization of a highly conserved binding site of Mlh1 required for exonuclease I-dependent mismatch repair. *Mol. Cell. Biol.* 29:907–18
42. Doe C, Dixon J, Osman F, Whitby M. 2000. Partial suppression of the fission yeast *rqh1*$^{-}$ phenotype by expression of a bacterial Holliday junction resolvase. *EMBO J.* 19:2751–62
43. Dutertre S, Ababou M, Onclercq R, Delic J, Chatton B, et al. 2000. Cell cycle regulation of the endogenous wild type Bloom's syndrome DNA helicase. *Oncogene* 19:2731–38
44. Ellis NA, Groden J, Ye TZ, Straughen J, Lennon DJ, et al. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83:655–66
45. Frei C, Gasser SM. 2000. The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci. *Genes Dev.* 14:81–96
46. Fricke W, Kaliraman V, Brill S. 2001. Mapping the DNA topoisomerase III binding domain of the Sgs1 DNA helicase. *J. Biol. Chem.* 276:8848–55
47. Fukuchi K, Martin GM, Monnat RJ Jr. 1989. Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proc. Natl. Acad. Sci. USA* 86:5893–97
48. Fukuchi K, Tanaka K, Nakura J, Kumahara Y, Uchida T, Okada Y. 1985. Elevated spontaneous mutation rate in SV40-transformed Werner syndrome fibroblast cell lines. *Somat. Cell Mol. Genet.* 11:303–8
49. Gangloff S, de Massy B, Arthur L, Rothstein R, Fabre F. 1999. The essential role of yeast topoisomerase III in meiosis depends on recombination. *EMBO J.* 18:1701–11
50. Gangloff S, McDonald J, Bendixen C, Arthur L, Rothstein R. 1994. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* 14:8391–98
51. German J. 1993. Bloom syndrome: a mendelian prototype of somatic mutational disease. *Medicine* 72:393–406
52. German J, Archibald R, Bloom D. 1965. Chromosomal breakage in a rare and probably genetically determined syndrome of man. *Science* 148:506–7
53. German J, Sanz MM, Ciocci S, Ye TZ, Ellis NA. 2007. Syndrome-causing mutations of the BLM gene in persons in the Bloom's syndrome registry. *Hum. Mutat.* 28:743–53
54. Gharibyan V, Youssoufian H. 1999. Localization of the Bloom syndrome helicase to punctate nuclear structures and the nuclear matrix and regulation during the cell cycle: comparison with the Werner's syndrome helicase. *Mol. Carcinog.* 26:261–73
55. Gilbertson L, Stahl F. 1996. A test of the double-strand break repair model for meiotic recombination in *Saccharomyces cerevisiae*. *Genetics* 144:27–41
56. Gorbalenya AE, Koonin EV. 1993. Helicases: amino acid sequence comparisons and structure-function relationships. *Curr. Opin. Struct. Biol.* 3:419–29
57. Goss KH, Risinger MA, Kordich JJ, Sanz MM, Straughen JE, et al. 2002. Enhanced tumor formation in mice heterozygous for Blm mutation. *Science* 297:2051–53

58. Goto M. 1997. Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. *Mech. Ageing Dev.* 98:239–54
59. Grabowski MM, Svrzikapa N, Tissenbaum HA. 2005. Bloom syndrome ortholog HIM-6 maintains genomic stability in *C. elegans*. *Mech. Ageing Dev.* 126:1314–21
60. Gravel S, Chapman JR, Magill C, Jackson SP. 2009. DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev.* 22:2767–72
61. Harmon F, Kowalczykowski S. 1998. RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. *Genes Dev.* 12:1134–44
62. Hartung F, Puchta H. 2006. The RecQ gene family in plants. *J. Plant Physiol.* 163:287–96
63. Hodgkin J, Horvitz H, Brenner S. 1979. Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* 91:67–94
64. Hoki Y, Araki R, Fujimori A, Ohhata T, Koseki H, et al. 2003. Growth retardation and skin abnormalities of the Recql4-deficient mouse. *Hum. Mol. Genet.* 12:2293–99
65. Hu Y, Raynard S, Sehorn MG, Lu X, Bussen W, et al. 2007. RECQL5/Recql5 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. *Genes Dev.* 21:3073–84
66. Huang S, Lee L, Hanson NB, Lenaerts C, Hoehn H, et al. 2006. The spectrum of WRN mutations in Werner syndrome patients. *Hum. Mutat.* 27:558–67
67. Huber MD, Lee DC, Maizels N. 2002. G4 DNA unwinding by BLM and Sgs1p: substrate specificity and substrate-specific inhibition. *Nucleic Acids Res.* 30:3954–61
68. Hyun M, Bohr V, Ahn B. 2008. Biochemical characterization of the WRN-1 RecQ helicase of *Caenorhabditis elegans*. *Biochemistry* 47:7583–93
69. Ira G, Malkova A, Liberi G, Foiani M, Haber J. 2003. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* 115:401–11
70. Jessop L, Lichten M. 2008. Mus81/Mms4 endonuclease and Sgs1 helicase collaborate to ensure proper recombination intermediate metabolism during meiosis. *Mol. Cell* 31:313–23
71. Jessop L, Rockmill B, Roeder G, Lichten M. 2006. Meiotic chromosome synapsis-promoting proteins antagonize the anti-crossover activity of Sgs1. *PLoS Genet.* 2(9):e155
72. Jiao R, Bachrati C, Pedrazzi G, Kuster P, Petkovic M, et al. 2004. Physical and functional interaction between the Bloom's syndrome gene product and the largest subunit of chromatin assembly factor 1. *Mol. Cell. Biol.* 24:4710–19
73. Johnson F, Lombard D, Neff N, Mastrangelo M, Dewolf W, et al. 2000. Association of the Bloom syndrome protein with topoisomerase IIIalpha in somatic and meiotic cells. *Cancer Res.* 60:1162–67
74. Kamath-Loeb A, Johansson E, Burgers P, Loeb L. 2000. Functional interaction between the Werner syndrome protein and DNA polymerase delta. *Proc. Natl. Acad. Sci. USA* 97:4603–8
75. Karow J, Constantinou A, Li J, West S, Hickson I. 2000. The Bloom's syndrome gene product promotes branch migration of Holliday junctions. *Proc. Natl. Acad. Sci. USA* 97:6504–8
76. Karow JK, Newman RH, Freemont PS, Hickson ID. 1999. Oligomeric ring structure of the Bloom's syndrome helicase. *Curr. Biol.* 9:597–600
77. Kerrest A, Anand RP, Sundararajan R, Bermejo R, Liberi G, et al. 2009. *SRS2* and *SGS1* prevent chromosomal breaks and stabilize triplet repeats by restraining recombination. *Nat. Struct. Mol. Biol.* 16:159–67
78. Khakhar RR, Cobb JA, Bjergbaek L, Hickson ID, Gasser SM. 2003. RecQ helicases: multiple roles in genome maintenance. *Trends Cell Biol.* 13:493–501
79. Kitao S, Ohsugi I, Ichikawa K, Goto M, Furuichi Y, Shimamoto A. 1998. Cloning of two new human helicase genes of the RecQ family: biological significance of multiple species in higher eukaryotes. *Genomics* 54:443–52
80. Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, et al. 1999. Mutations in RECQL4 cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* 22:82–84
81. Kobbe D, Blanck S, Demand K, Focke M, Puchta H. 2008. AtRECQ2, a RecQ helicase homologue from *Arabidopsis thaliana*, is able to disrupt various recombinogenic DNA structures in vitro. *Plant J.* 55:397–405

82. Krejci L, Van Komen S, Li Y, Villemain J, Reddy MS, et al. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* 423:305–9
83. Kusano K, Johnson-Schlitz DM, Engels WR. 2001. Sterility of *Drosophila* with mutations in the Bloom syndrome gene: complementation by Ku70. *Science* 291:2600–2
84. Liao S, Toczylowski T, Yan H. 2008. Identification of the *Xenopus* DNA2 protein as a major nuclease for the 5′-3′ strand-specific processing of DNA ends. *Nucleic Acids Res.* 36(19):6091–100
85. Langland G, Kordich J, Creaney J, Goss K, Lillard-Wetherell K, et al. 2001. The Bloom's syndrome protein (BLM) interacts with MLH1 but is not required for DNA mismatch repair. *J. Biol. Chem.* 276:30031–35
86. Larrivee M, LeBel C, Wellinger RJ. 2004. The generation of proper constitutive G-tails on yeast telomeres is dependent on the MRX complex. *Genes Dev.* 18:1391–96
87. Lebel M, Leder P. 1998. A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc. Natl. Acad. Sci. USA* 95:13097–102
88. Lebel M, Spillare EA, Harris CC, Leder P. 1999. The Werner syndrome gene product co-purifies with the DNA replication complex and interacts with PCNA and topoisomerase I. *J. Biol. Chem.* 274:37795–99
89. Lengsfeld BM, Rattray AJ, Bhaskara V, Ghirlando R, Paull TT. 2007. Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. *Mol. Cell* 28:638–51
90. LeRoy G, Carroll R, Kyin S, Seki M, Cole M. 2005. Identification of RecQL1 as a Holliday junction processing enzyme in human cell lines. *Nucleic Acids Res.* 33:6251–57
91. Li D, Frazier M, Evans DB, Hess KR, Crane CH, et al. 2006. Single nucleotide polymorphisms of RecQ1, RAD54L, and ATM genes are associated with reduced survival of pancreatic cancer. *J. Clin. Oncol.* 24:1720–28
92. Li JL, Harrison RJ, Reszka AP, Brosh RM Jr, Bohr VA, et al. 2001. Inhibition of the Bloom's and Werner's syndrome helicases by G-quadruplex interacting ligands. *Biochemistry* 40:15194–202
93. Liberi G, Maffioletti G, Lucca C, Chiolo I, Baryshnikova A, et al. 2005. Rad51-dependent DNA structures accumulate at damaged replication forks in Sgs1 mutants defective in the yeast ortholog of BLM RecQ helicase. *Genes Dev.* 19:339–50
94. Lisby M, Rothstein R. 2009. Choreography of recombination proteins during the DNA damage response. *DNA Repair* (*Amst*) 8:1068–76
95. Liu Z, Macias M, Bottomley M, Stier G, Linge J, et al. 1999. The three-dimensional structure of the HRDC domain and implications for the Werner and Bloom syndrome proteins. *Structure* 7:1557–66
96. Lonn U, Lonn S, Nylen U, Winblad G, German J. 1990. An abnormal profile of DNA replication intermediates in Bloom's syndrome. *Cancer Res.* 50:3141–45
97. Louis E, Borts R. 2003. Meiotic recombination: too much of a good thing? *Curr. Biol.* 13:R953–55
98. Lu CY, Tsai CH, Brill SJ, Teng SC. 2009. Sumoylation of the BLM ortholog, Sgs1, promotes telomere-telomere recombination in budding yeast. *Nucleic Acids Res.* 38(2):488–98
99. Luo G, Santoro IM, McDaniel LD, Nishijima I, Mills M, et al. 2000. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. *Nat. Genet.* 26:424–29
100. Lydeard JR, Lipkin-Moore Z, Jain S, Eapen VV, Haber JE. 2010. Sgs1 and Exo1 redundantly inhibit break-induced replication and de novo telomere addition at broken chromosome ends. *PLoS Genet.* 6(5):e100097
101. Mann MB, Hodges CA, Barnes E, Vogel H, Hassold TJ, Luo G. 2005. Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund-Thomson syndrome. *Hum. Mol. Genet.* 14:813–25
102. Mimitou EP, Symington LS. 2008. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* 455(7214):770–75
103. Miozzo M, Castorina P, Riva P, Dalpra L, Fuhrman Conti AM, et al. 1998. Chromosomal instability in fibroblasts and mesenchymal tumors from 2 sibs with Rothmund-Thomson syndrome. *Int. J. Cancer* 77:504–10
104. Miyajima A, Seki M, Onoda F, Shiratori M, Odagiri N, et al. 2000. Sgs1 helicase activity is required for mitotic but apparently not for meiotic functions. *Mol. Cell. Biol.* 20:6399–409

105. Moens PB, Freire R, Tarsounas M, Spyropoulos B, Jackson SP. 2000. Expression and nuclear localization of BLM, a chromosome stability protein mutated in Bloom's syndrome, suggest a role in recombination during meiotic prophase. *J. Cell Sci.* 113(4):663–72
106. Mohaghegh P, Karow JK, Brosh RM Jr, Bohr VA, Hickson ID. 2001. The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. *Nucleic Acids Res.* 29:2843–49
107. Mullen J, Kaliraman V, Brill S. 2000. Bipartite structure of the *SGS1* DNA helicase in *Saccharomyces cerevisiae*. *Genetics* 154:1101–14
108. Mullen J, Nallaseth F, Lan Y, Slagle C, Brill S. 2005. Yeast Rmi1/Nce4 controls genome stability as a subunit of the Sgs1-Top3 complex. *Mol. Cell. Biol.* 25:4476–87
109. Muzzolini L, Beuron F, Patwardhan A, Popuri V, Cui S, et al. 2007. Different quaternary structures of human RECQ1 are associated with its dual enzymatic activity. *PLoS Biol.* 5:e20
110. Nasmyth KA. 1982. Molecular genetics of yeast mating type. *Annu. Rev. Genet.* 16:439–500
111. Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC. 2008. Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proc. Natl. Acad. Sci. USA* 105:16906–11
112. Niu H, Chung W-H, Zhu Z, Kwon Y, Zhao W, et al. 2010. Mechanism of the ATP-dependent DNA end resection machinery from *S. cerevisiae*. *Nature*. In press
113. Oh SD, Lao JP, Hwang PY, Taylor AF, Smith GR, Hunter N. 2007. BLM ortholog, Sgs1, prevents aberrant crossing-over by suppressing formation of multichromatid joint molecules. *Cell* 130:259–72
114. Oh S, Lao J, Taylor A, Smith G, Hunter N. 2008. RecQ helicase, Sgs1, and XPF family endonuclease, Mus81-Mms4, resolve aberrant joint molecules during meiotic recombination. *Mol. Cell* 31:324–36
115. Opresko P, Sowd G, Wang H. 2009. The Werner syndrome helicase/exonuclease processes mobile D-loops through branch migration and degradation. *PLoS ONE* 4:e4825
116. Pedruzzi I, Burckert N, Egger P, De Virgilio C. 2000. *Saccharomyces cerevisiae* Ras/cAMP pathway controls post-diauxic shift element-dependent transcription through the zinc finger protein Gis1. *EMBO J.* 19:2569–79
117. Pike A, Shrestha B, Popuri V, Burgess-Brown N, Muzzolini L, et al. 2009. Structure of the human RECQ1 helicase reveals a putative strand-separation pin. *Proc. Natl. Acad. Sci. USA* 106:1039–44
118. Plank J, Wu J, Hsieh T. 2006. Topoisomerase IIIalpha and Bloom's helicase can resolve a mobile double Holliday junction substrate through convergent branch migration. *Proc. Natl. Acad. Sci. USA* 103:11118–23
119. Poot M, Hoehn H, Runger TM, Martin GM. 1992. Impaired S-phase transit of Werner syndrome cells expressed in lymphoblastoid cell lines. *Exp. Cell Res.* 202:267–73
120. Popuri V, Bachrati C, Muzzolini L, Mosedale G, Costantini S, et al. 2008. The human Recq helicases, Blm and Recq1, display distinct DNA substrate specificities. *J. Biol. Chem.* 283:17766–76
121. Puranam KL, Blackshear PJ. 1994. Cloning and characterization of RECQL, a potential human homologue of the *Escherichia coli* DNA helicase RecQ. *J. Biol. Chem.* 269:29838–45
122. Raynard S, Bussen W, Sung P. 2006. A double Holliday junction dissolvasome comprising BLM, topoisomerase IIIalpha, and BLAP75. *J. Biol. Chem.* 281:13861–64
123. Raynard S, Zhao W, Bussen W, Lu L, Ding Y, et al. 2008. Functional Role of BLAP75 in BLM-topoisomerase III{alpha}-dependent Holliday junction processing. *J. Biol. Chem.* 283:15701–8
124. Robert T, Dervins D, Fabre F, Gangloff S. 2006. Mrc1 and Srs2 are major actors in the regulation of spontaneous crossover. *EMBO J.* 25:2837–46
125. Rockmill B, Fung JC, Branda SS, Roeder GS. 2003. The Sgs1 helicase regulates chromosome synapsis and meiotic crossing over. *Curr. Biol.* 13:1954–62
126. Rockmill B, Voelkel-Meiman K, Roeder GS. 2006. Centromere-proximal crossovers are associated with precocious separation of sister chromatids during meiosis in *Saccharomyces cerevisiae*. *Genetics* 174:1745–54
127. Rog O, Miller KM, Ferreira MG, Cooper JP. 2009. Sumoylation of RecQ helicase controls the fate of dysfunctional telomeres. *Mol. Cell* 33:559–69
128. Saffi J, Feldmann H, Winnacker E, Henriques J. 2001. Interaction of the yeast Pso5/Rad16 and Sgs1 proteins: influences on DNA repair and aging. *Mutat. Res.* 486:195–206
129. Salk D, Bryant E, Au K, Hoehn H, Martin GM. 1981. Systematic growth studies, cocultivation, and cell hybridization studies of Werner syndrome cultured skin fibroblasts. *Hum. Genet.* 58:310–16

130. Seki M, Otsuki M, Ishii Y, Tada S, Enomoto T. 2008. RecQ family helicases in genome stability. *Cell Cycle* 7:2472–78
131. Seki M, Yanagisawa J, Kohda T, Sonoyama T, Ui M, Enomoto T. 1994. Purification of two DNA-dependent adenosinetriphosphatases having DNA helicase activity from HeLa cells and comparison of the properties of the two enzymes. *J. Biochem.* 115:523–31
132. Sengupta S, Linke SP, Pedeux R, Yang Q, Farnsworth J, et al. 2003. BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. *EMBO J.* 22:1210–22
133. Sengupta S, Robles AI, Linke SP, Sinogeeva NI, Zhang R, et al. 2004. Functional interaction between BLM helicase and 53BP1 in a Chk1-mediated pathway during S-phase arrest. *J. Cell Biol.* 166:801–13
134. Shen JC, Gray MD, Oshima J, Loeb LA. 1998. Characterization of Werner syndrome protein DNA helicase activity: directionality, substrate dependence and stimulation by replication protein A. *Nucleic Acids Res.* 26:2879–85
135. Shimamoto A, Nishikawa K, Kitao S, Furuichi Y. 2000. Human RecQ5beta, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3alpha and 3beta. *Nucleic Acids Res.* 28:1647–55
136. Siitonen HA, Kopra O, Kaariainen H, Haravuori H, Winter RM, et al. 2003. Molecular defect of RAPADILINO syndrome expands the phenotype spectrum of RECQL diseases. *Hum. Mol. Genet.* 12:2837–44
137. Siitonen HA, Sotkasiira J, Biervliet M, Benmansour A, Capri Y, et al. 2009. The mutation spectrum in RECQL4 diseases. *Eur. J. Hum. Genet.* 17:151–58
138. Singh T, Ali A, Busygina V, Raynard S, Fan Q, et al. 2008. BLAP18/RMI2, a novel OB-fold-containing protein, is an essential component of the Bloom helicase-double Holliday junction dissolvasome. *Genes Dev.* 22:2856–68
139. Spillare EA, Robles AI, Wang XW, Shen JC, Yu CE, et al. 1999. p53-mediated apoptosis is attenuated in Werner syndrome cells. *Genes Dev.* 13:1355–60
140. Sun H, Bennett RJ, Maizels N. 1999. The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs. *Nucleic Acids Res.* 27:1978–84
141. Tahara H, Kusunoki M, Yamanaka Y, Matsumura S, Ide T. 2005. G-tail telomere HPA: simple measurement of human single-stranded telomeric overhangs. *Nat. Methods* 2:829–31
142. Theobald D, Mitton-Fry R, Wuttke D. 2003. Nucleic acid recognition by OB-fold proteins. *Annu. Rev. Biophys. Biomol. Struct.* 32:115–33
143. Tronnersjo S, Hanefalk C, Balciunas D, Hu G, Nordberg N, et al. 2007. The jmjN and jmjC domains of the yeast zinc finger protein Gis1 interact with 19 proteins involved in transcription, sumoylation and DNA repair. *Mol. Genet. Genomics* 277:57–70
144. Tsukada Y, Fang J, Erdjument-Bromage H, Warren M, Borchers C, et al. 2006. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 439:811–16
145. Van Brabant AJ, Ye T, Sanz M, German IJ, Ellis NA, Holloman WK. 2000. Binding and melting of D-loops by the Bloom syndrome helicase. *Biochemistry* 39:14617–25
146. Van Maldergem L, Siitonen HA, Jalkh N, Chouery E, De Roy M, et al. 2006. Revisiting the craniosynostosis-radial ray hypoplasia association: Baller-Gerold syndrome caused by mutations in the RECQL4 gene. *J. Med. Genet.* 43:148–52
147. Veaute X, Jeusset J, Soustelle C, Kowalczykowski SC, Le Cam E, Fabre F. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* 423:309–12
148. Versini G, Comet I, Wu M, Hoopes L, Schwob E, Pasero P. 2003. The yeast Sgs1 helicase is differentially required for genomic and ribosomal DNA replication. *EMBO J.* 22:1939–49
149. Vindigni A, Hickson I. 2009. RecQ helicases: multiple structures for multiple functions? *HFSP J.* 3:153–64
150. von Kobbe C, Karmakar P, Dawut L, Opresko P, Zeng X, et al. 2002. Colocalization, physical, and functional interaction between Werner and Bloom syndrome proteins. *J. Biol. Chem.* 277:22035–44
151. Wallis JW, Chrebet G, Brodsky G, Rolfe M, Rothstein R. 1989. A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell* 58:409–19

152. Wang T, Kung W. 2002. Supercomplex formation between Mlh1-Mlh3 and Sgs1-Top3 heterocomplexes in meiotic yeast cells. *Biochem. Biophys. Res. Commun.* 296:949–53
153. Wang XW, Tseng A, Ellis NA, Spillare EA, Linke SP, et al. 2001. Functional interaction of p53 and BLM DNA helicase in apoptosis. *J. Biol. Chem.* 276:32948–55
154. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* 14:927–39
155. Watt P, Hickson I, Borts R, Louis E. 1996. *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics* 144:935–45
156. Watt P, Louis E, Borts R, Hickson I. 1995. Sgs1: a eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. *Cell* 81:253–60
157. White CI, Haber JE. 1990. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. *EMBO J.* 9:663–73
158. Wu L, Bachrati CZ, Ou J, Xu C, Yin J, et al. 2006. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. *Proc. Natl. Acad. Sci. USA* 103:4068–73
159. Wu L, Chan K, Ralf C, Bernstein D, Garcia P, et al. 2005. The HRDC domain of BLM is required for the dissolution of double Holliday junctions. *EMBO J.* 24:2679–87
160. Wu L, Davies S, Levitt N, Hickson I. 2001. Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51. *J. Biol. Chem.* 276:19375–81
161. Wu L, Davies SL, North PS, Goulaouic H, Riou JF, et al. 2000. The Bloom's syndrome gene product interacts with topoisomerase III. *J. Biol. Chem.* 275:9636–44
162. Wu L, Hickson I. 2003. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 426:870–74
163. Xu D, Guo R, Sobeck A, Bachrati C, Yang J, et al. 2008. RMI, a new OB-fold complex essential for Bloom syndrome protein to maintain genome stability. *Genes Dev.* 22:2843–55
164. Xu H, Deprez E, Zhang A, Tauc P, Ladjimi M, et al. 2003. The *Escherichia coli* RecQ helicase functions as a monomer. *J. Biol. Chem.* 278:34925–33
165. Xu X, Rochette PJ, Feyissa EA, Su TV, Liu Y. 2009. MCM10 mediates RECQ4 association with MCM2–7 helicase complex during DNA replication. *EMBO J.* 28:3005–14
166. Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, et al. 2006. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell* 125:483–95
167. Yang Q, Zhang R, Wang X, Spillare E, Linke S, et al. 2002. The processing of Holliday junctions by BLM and WRN helicases is regulated by p53. *J. Biol. Chem.* 277:31980–87
168. Yin J, Sobeck A, Xu C, Meetei AR, Hoatlin M, et al. 2005. BLAP75, an essential component of Bloom's syndrome protein complexes that maintain genome integrity. *EMBO J.* 24:1465–76
169. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, et al. 1996. Positional cloning of the Werner's syndrome gene. *Science* 272:258–62
170. Zhang X, Dou S, Xie P, Hu J, Wang P, Xi X. 2006. *E. coli* RecQ is a rapid, efficient and monomeric helicase. *J. Biol. Chem.* 281:12655–63
171. Zhong S, Hu P, Ye T, Stan R, Ellis N, Pandolfi P. 1999. A role for PML and the nuclear body in genomic stability. *Oncogene* 18:7941–47
172. Zhu Z, Chung WH, Shim EY, Lee SE, Ira G. 2008. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* 134:981–94