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Structure and Function of RecQ DNA Helicases

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RecQ family helicases play important roles in coordinating genome maintenance pathways in living cells. In the absence of functional RecQ proteins, cells exhibit a variety of phenotypes, including increased mitotic recombination, elevated chromosome missegregation, hypersensitivity to DNA-damaging agents, and defects in meiosis. Mutations in three of the five human RecQ family members give rise to genetic disorders associated with a predisposition to cancer and premature aging, highlighting the importance of RecQ proteins and their cellular activities for human health. Current evidence suggests that RecQ proteins act at multiple steps in DNA replication, including stabilization of replication forks and removal of DNA recombination intermediates, in order to maintain genome integrity. The cellular basis of RecQ helicase function may be explained through interactions with multiple components of the DNA replication and recombination machinery. This review focuses on biochemical and structural aspects of the RecQ helicases and how these features relate to their known cellular function, specifically in preventing excessive recombination.

Keywords DNA replication, DNA recombination, DNA repair, Bloom's syndrome, Werner's syndrome, Rothmund-Thomson syndrome

The importance of genome stability is evidenced by the many mechanisms used by the cell to protect its genetic material and by the difficulties cells face when these mechanisms break down. Many human disorders have been linked to genome instability caused by defects in proteins involved in DNA replication, recombination, and repair. One of the most significant findings in this field is the seemingly symbiotic relationship between DNA replication and recombination pathways. In particular, replication forks stalled by damage to the genome can be rescued through recombinational events that ensure completion of the DNA duplication process (Cox *et al.*, 2000). This induction of recombination is advantageous under some conditions, but excessive recombination is clearly deleterious to the cell. Several proteins that function at the interface of replication and recombination have been identified, and particular attention has been given to the RecQ family of DNA helicases, where defects in human members of this family give rise to debilitating genetic disorders.

The RecQ family of DNA helicases is named after the first member of the family to be identified, the *recQ* gene of *Escherichia coli*, which was identified 20 years ago in a screen for mutations that provide resistance to thymine starvation (Nakayama *et al.*, 1984). Studies in *E. coli* suggested that RecQ acts both as a suppressor of illegitimate recombination (Hanada *et al.*, 1997) and as an initiator of homologous recombination via the RecF recombinational pathway (Ryder *et al.*, 1994). This dualism in recombination pathways is also observed with eukaryotic RecQ helicases—under most circumstances the RecQ proteins act as antirecombinases, but RecQ proteins can also promote recombination, making simplistic interpretation of their functions difficult. A single RecQ homolog has been identified in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, namely Sgs1 and Rqh1 proteins, respectively (Gangloff *et al.*, 1994; Stewart *et al.*, 1997; see Figure 1). Higher eukaryotes typically encode multiple *recQ* genes, and five members have been identified in humans.

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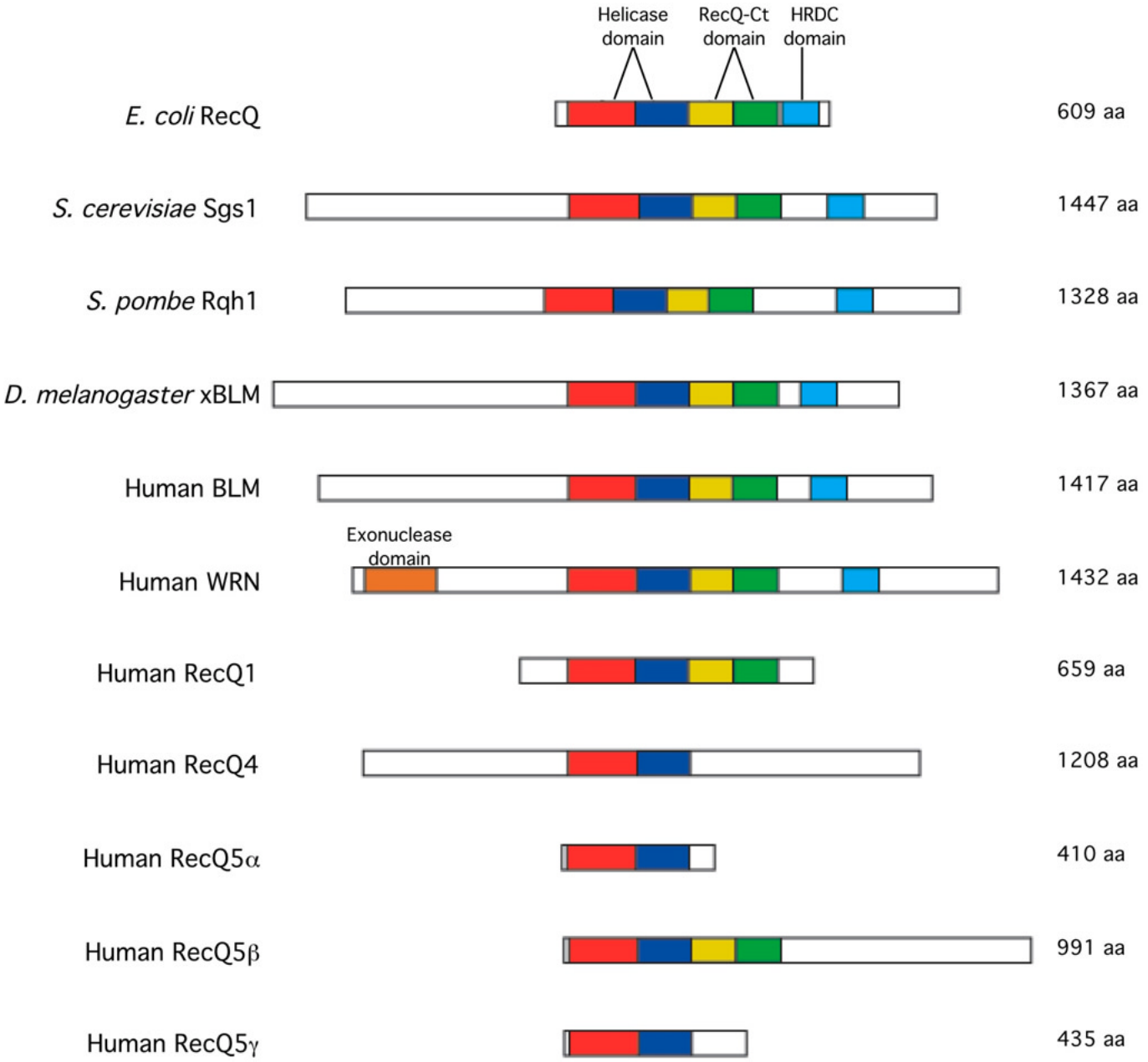

FIG. 1. Schematic diagram of selected members of the RecQ family of DNA helicases. The color scheme matches the scheme shown in Figure 3 of the structures of the protein domains. Helicase subdomains are shown in red and blue, and the RecQ-Ct domain is divided into a Zn^{2+}-binding subdomain (yellow) and a WH domain (green). The HRDC domain is depicted in cyan, and the exonuclease domain of WRN is shown in brown.

Mutations in three of the five human *recQ* homologs have been linked to rare autosomal-recessive disorders; the *BLM, WRN*, and *RECQ4* genes are linked to Bloom's syndrome (BS), Werner's syndrome (WS), and Rothmund-Thomson syndrome (RTS), respectively (Ellis *et al.*, 1995; Kitao *et al.*, 1999; Yu *et al.*, 1996). While the cellular defects observed in these three syndromes are distinct, there are several common abnormalities, including chromosomal instability, a predisposition to cancer, and, in the case of Werner and Rothmund-Thomson syndrome, premature aging. A detailed discussion of these syndromes and the role of *BLM*, *WRN*, and *RECQ4* as "caretaker genes" in preventing tumorigenesis can be found in recent reviews (Harrigan & Bohr, 2003; Hickson, 2003). A fourth disease, RAPADILINO syndrome, which shares some clinical characteristics with RTS, has also been linked recently

to a subset of mutations of *RECQ4* (Siitonen *et al.*, 2003). Thus, the activities of RecQ proteins can have an important impact on human health.

A significant size difference is notable between the *E. coli* RecQ protein and many of the eukaryotic RecQ family members. Whereas *E. coli* RecQ is only 609 amino acids in length, the eukaryotic proteins are often more than twice as large (see Figure 1). The large size difference reflects the presence of additional domains in the eukaryotic RecQ proteins that either encode additional enzymatic activities (*e.g.*, the N-terminus of human WRN protein encodes an exonuclease activity) or else have been shown to mediate interactions with other protein partners. The importance of these interactions can be implied from studies on the human RecQ proteins. Although the five human RecQ proteins share very similar helicase domains, they are not redundant in function, and defects in the genes exhibit different phenotypes. It seems likely that protein interactions play a major role in directing the RecQ helicases to different activities in the cell.

BIOCHEMICAL ACTIVITIES OF RecQ PROTEINS

The *E. coli* RecQ protein was the first RecQ helicase to be purified and was shown to catalyze the ATP-dependent unwinding of double-stranded DNA (dsDNA) in a 3′ to 5′ direction *in vitro*. This feature is apparently conserved among all RecQ helicases (Bennett *et al.*, 1998; Gray *et al.*, 1997; Karow *et al.*, 1997; Suzuki *et al.*, 1997; Umezu *et al.*, 1990; see Figure 2. Directionality is defined by the direction a helicase moves along the flanking single-stranded DNA (ssDNA) in a partial-duplex DNA structure). In the case of Sgs1, the 3′ to 5′ polarity of DNA unwinding is a direct consequence of Sgs1 binding at the ssDNA/dsDNA junction and its recognition of the polarity of ssDNA at the junction (Bennett *et al.*, 1998). The processivity of DNA unwinding by RecQ helicases is relatively low, particularly in the case of WRN protein, which is unable to unwind duplex regions longer than 40 basepairs (bp) in length. The presence of accessory factors such as ssDNA-binding proteins can significantly enhance RecQ helicase processivity so that, for example, fragments up to 558 bp can be unwound by Sgs1 helicase in the presence of replication protein A (RPA) (Bennett *et al.*, 1998; Brosh *et al.*, 2000; Gray *et al.*, 1997; Karow *et al.*, 1997; Suzuki *et al.*, 1997; Umezu & Nakayama, 1993).

Additional studies indicate that many RecQ proteins exhibit preferential activity on specialized DNA structures. Preferred substrates are branched DNA structures, including forked structures that mimic replication forks, and synthetic 4-way junctions that mimic Holliday junctions (HJs; see Figure 2). The human BLM and WRN proteins also promote branch migration of HJs formed as recombination intermediates made by RecA protein over several thousand

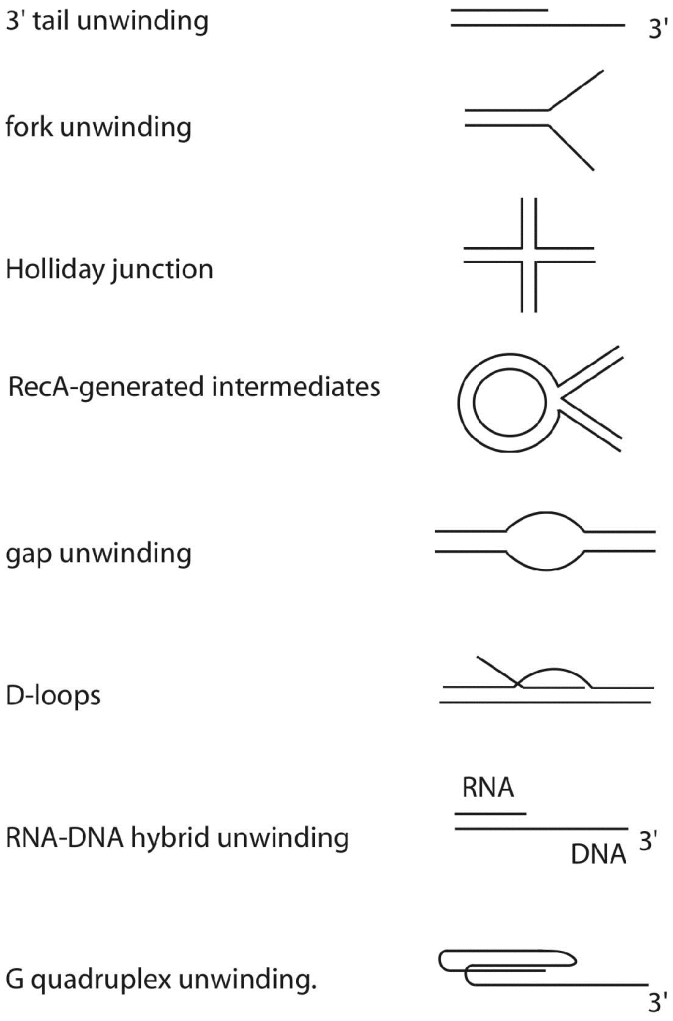

FIG. 2. DNA substrates for RecQ helicase activity. Several branched DNA structures are favored substrates for unwinding by RecQ. Note that many of the structures require a flanking 3′ ssDNA tail for efficient recognition by RecQ. A notable exception is the 4-way junction recognized in synthetic Holliday junctions and RecA-generated intermediates.

basepairs in length (Constantinou *et al.*, 2000; Harmon & Kowalczykowski, 1998; Karow *et al.*, 2000).

RecQ helicases are also active in unwinding a number of other substrates including gapped DNA, D-loop structures, RNA–DNA hybrids, and triplex DNA (Bennett *et al.*, 1998; Brosh *et al.*, 2001a; Machwe *et al.*, 2002; Mohaghegh *et al.*, 2001; Orren *et al.*, 2002; van Brabant *et al.*, 2000; see Figure 2). In addition, the *E. coli* RecQ protein is unique among characterized RecQ helicases in being able to unwind fully duplex DNA molecules. This ability may be important for *E. coli* RecQ to generate ssDNA tails for the initiation of DNA strand exchange by the RecA protein (Harmon & Kowalczykowski, 1998). These studies

provided early clues that some of the targets of RecQ proteins would be intermediates in replication and recombination.

Sgs1, BLM, and WRN proteins are also efficient at unwinding G-quadruplex (G4) DNA (Han *et al.*, 2000; Huber *et al.*, 2002; Mohaghegh *et al.*, 2001; Sun *et al.*, 1998, 1999). G4 structures are highly stable and have been implicated in telomere function as G-rich strands are present in telomere repeats. As discussed below, G4-containing DNA might also act as "roadblocks" during replication, where their removal could be necessary for progression of the replication fork. The ability to unwind G4 DNA appears to be unique to RecQ helicases, as other DNA helicases such as *E. coli* RecBCD are unable to unwind such DNA structures (Sun *et al.*, 1998). Similar to the unwinding of duplex DNA, the unwinding of G4 DNA by RecQ helicases requires that the G4 DNA include a free 3′ single-stranded tail (Mohaghegh *et al.*, 2001; Sun *et al.*, 1998).

The human WRN and *X. leavis* FFA-1 proteins are unique among the RecQ family in that they also encode a 3′ to 5′ exonuclease activity at their N-termini (Shen *et al.*, 1998; Yan *et al.*, 1998; see Figure 1). The helicase and exonuclease activities in WRN are physically separable; recombinant fragments comprising either the helicase domain or the exonuclease domain can be expressed and are functional for helicase or exonuclease activity, respectively (Machwe *et al.*, 2002; Xue *et al.*, 2002). However, there is evidence that these domains work in concert in the full-length WRN protein (Machwe *et al.*, 2002; Opresko *et al.*, 2001). While other RecQ helicases do not contain an intrinsic nuclease activity, many have been shown to interact functionally with DNA nucleases in the cell (*e.g.*, RecQ functions with the RecJ exonuclease in *E. coli*; Courcelle & Hanawalt, 1999); thus the combination of RecQ helicase with a DNA nuclease appears to be a common feature of this family.

STRUCTURAL INSIGHTS INTO RecQ PROTEINS

As described above, RecQ DNA helicases have several domains with identified biochemical roles (Figure 1). The best conserved of these is the helicase domain, which catalyzes the DNA unwinding activity that is common to all RecQ family members. In most RecQ proteins the helicase domain is followed by RecQ C-terminal (RecQ-Ct) and "Helicase and RNase D C-terminal" (HRDC) domains, which are involved in DNA binding and in mediating interactions with other proteins. Biochemical and structural efforts have defined the boundaries of several domains in RecQ family helicases and, in some cases, have revealed their high-resolution structures (Figure 3). These studies have offered an intriguing view into the molecular mechanisms used by RecQ helicases. In this section, we will review recent structural insights into RecQ proteins with an emphasis on their links to biochemical roles of conserved domains in the enzyme family.

Helicase Domain of RecQ Proteins

Helicases use the energy derived from binding and hydrolysis of nucleoside triphosphates (NTPs) to catalyze unwinding of dsDNA or dsRNA. NTPase activity, and the conversion of the resulting energy into work to drive double-stranded nucleic acid unwinding, is coordinated by a series of seven sequence motifs (I, Ia, II, III, IV, V, and VI) that are hallmarks of superfamily-1 and -2 helicases (Gorbalenya & Koonin, 1993). An additional motif (motif 0) that is N-terminal to motif I is also conserved among RecQ family members (Bernstein & Keck, 2003). Crystal structures of a catalytic core fragment of *E. coli* RecQ, which includes its helicase and RecQ-Ct domains, have recently been determined (Bernstein *et al.*, 2003; Figure 3a). Coupled with a growing body of biochemical and genetic data, this structure helps form a mechanistic picture of RecQ-mediated DNA unwinding processes.

ATP Binding and Hydrolysis by RecQ Motifs 0, I, and II. Motif 0 was identified in *E. coli* RecQ as a sequence upstream of motif I that, when partially deleted, led to drastic reduction of ATPase activity (Bernstein & Keck, 2003). The motif sequence $Lx_3(F/Y/W)Gx_3F(R/K)x_2Q$ is highly conserved among bacterial and eukaryotic RecQ family members. In terms of its position upstream of motif I and conservation of key residues, this sequence also bears similarity to the *Q motif* ($Fx_{16}GFx_2PxPIQ$) that is present in RNA helicases (Tanner *et al.*, 2003). In these proteins, the Q motif forms a bipartite adenine-binding pocket in which the glutamine side-chain hydrogen bonds with the N6 and N7 atoms of the base, and an upstream phenylalanine provides base-stacking interactions. The presence of conserved aromatic and glutamine residues in motif 0 and the observation that deletion of part of this motif causes a loss of ATP function led to the idea that this region might play a similar role in ATP binding in RecQ proteins (Bernstein & Keck, 2003). This hypothesis was also consistent with mutagenesis data from BLM and Sgs1 proteins that indicated a critical role for motif 0 in their function. Mutation of the glutamine in human BLM motif 0 to arginine is sufficient to cause Bloom's syndrome (Ellis *et al.*, 1995) and the analogous mutation in murine BLM abolishes its ATPase and DNA-unwinding activities *in vitro* (Bahr *et al.*, 1998). In addition, mutation of the motif 0 glutamine in *S. cerevisiae* Sgs1 leads to hyperrecombination and DNA-damage sensitization phenotypes that are essentially the same as what is seen in *sgs1* cells (Onoda *et al.*, 2000).

The hypothesis that motif 0 provides an adenine-binding pocket was ultimately proven correct by solution of the

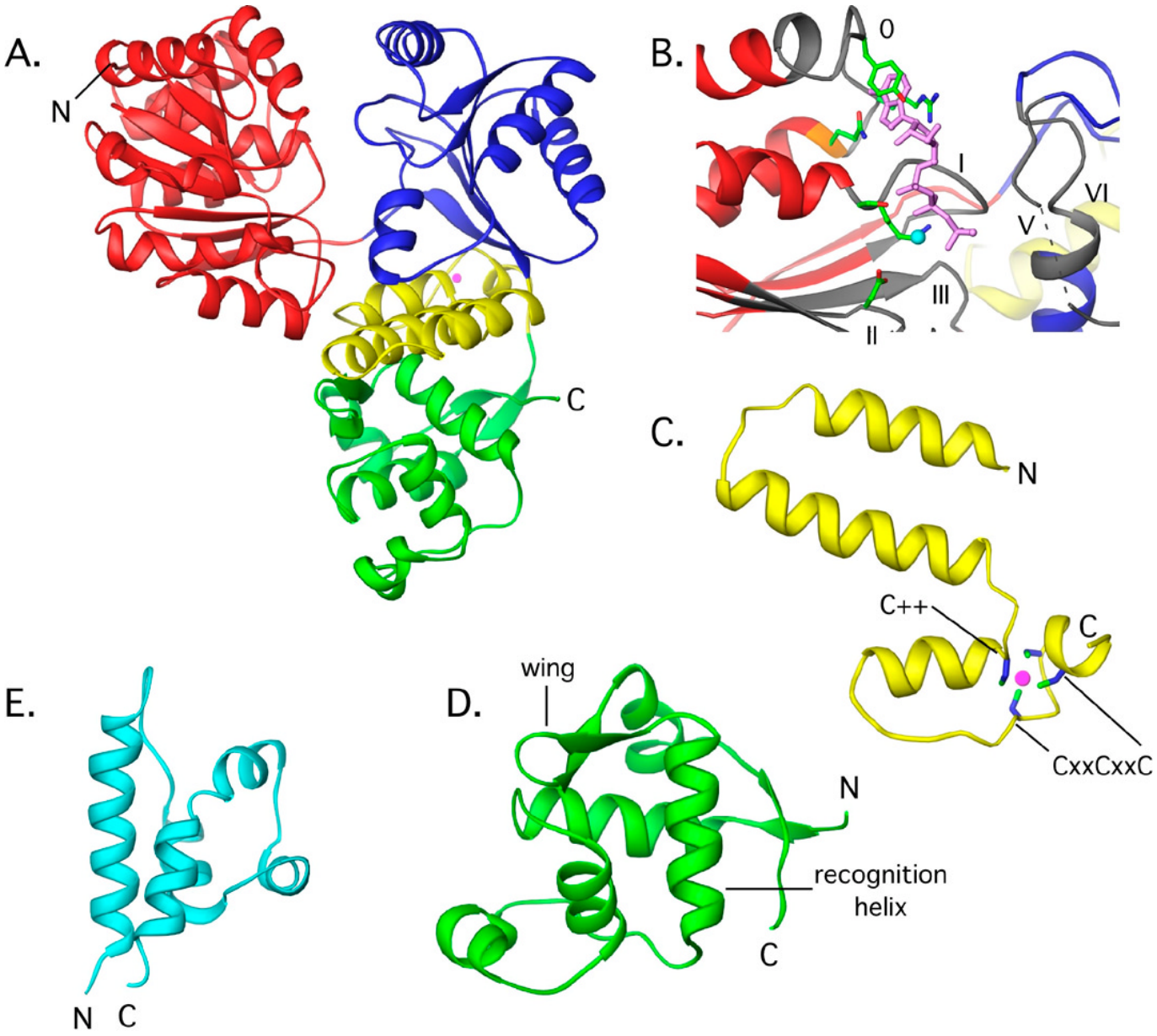

FIG. 3. High-resolution structures of protein domains in RecQ helicases. (a) X-ray crystal structure of the *E. coli* RecQ catalytic core (Bernstein *et al*., 2003). The domains are colored to reflect subdomains within the core; helicase lobes are colored in red and blue, the Zn^{2+}-binding RecQ-Ct subdomain is in yellow, and the WH RecQ-Ct subdomain is in green. A bound Zn^{2+} ion is shown as a magenta sphere. (b) Close-up of the ATP-binding site in the structure of the ATPγS-bound *E. coli* RecQ catalytic core. Coloring is same as in Figure 3a, except that several of helicase motifs are colored in grey and labeled (0, I, II, III, V, VI), and the ribbon diagram that corresponds to a key adenine-binding glutamine residue is colored orange. The ATPγS (ATP analog) is shown in pink, and a bound Mn^{2+} ion is shown as a cyan sphere. Side chains are shown for several key residues described in the text (motif 0: Tyrosine, Arginine, and Glutamine adenine-binding residues; motif I: Lysine and Serine phosphate- and metal-binding residues; motif II: Aspartate metal-binding residue). (c) Structure of just the Zn^{2+}-binding region of the RecQ-Ct domain from the *E. coli* RecQ catalytic core. Side chains of cysteine metal ligands are shown and labeled, and the bound Zn^{2+} is shown as a pink sphere. (d) Structure of just the WH domain of the RecQ-Ct domain from the *E. coli* RecQ catalytic core. Recognition helix and wing regions are labeled. (e) NMR structure of the *S. cerevisiae* Sgs1 HRDC domain (Liu *et al*., 1999).

structure of the catalytic core of *E. coli* RecQ bound to ATPγS, an ATP analog (Figure 3b; Bernstein *et al*., 2003). In this structure, the ATPγS adenine moiety is packed between tyrosine and arginine side chains from motif 0 and hydrogen bonds are formed between the N6 and N7 atoms of the adenine and the glutamine at the C-terminal end of the motif. Interestingly, although the glutamine and arginine residues of motif 0 are well conserved in RecQ helicases, the tyrosine is not, implying that differences in NTP binding may exist among RecQ family members.

As predicted by sequence similarity, the mode of adenine binding by *E. coli* RecQ is similar to that used in RNA helicases through their Q motif (Tanner *et al*., 2003). In addition, this adenine-binding arrangement shares strong similarity with PcrA DNA helicase, which packs an adenine moiety between tyrosine and arginine residues and also makes hydrogen bonds between an asparagine side chain and the N6 and N7 adenine atoms (Subramanya *et al.*, 1996; Velankar *et al.*, 1999). In contrast to the motif 0 and Q motif binding modes, however, the adenine-binding tyrosine side chain in PcrA is not encoded upstream of motif I.

Motifs I and II play clear roles in ATP binding and hydrolysis in RecQ helicases. These motifs (also known as Walker A and B boxes) have functions in NTP phosphate binding and hydrolysis that are conserved within a broad spectrum of NTP-dependent enzymes (Walker *et al.*, 1982). Motif I is typically defined by a $Gx_4GK(S/T)$ consensus sequence that interacts with phosphate moieties of an NTP via the lysine amino group. The S/T residue typically helps coordinate an active-site divalent metal ion that is important in hydrolysis of phosphoric acid anhydride bonds in NTPs. Motif I in RecQ family proteins (TGxGKS) differs slightly from the classical sequence (Morozov *et al.*, 1997) but appears to make the canonical phosphate and metal contacts (Figure 3b; Bernstein *et al*., 2003). Motif II, typically an acidic DExx sequence, supplies the major metal-coordinating element with the side chain of the aspartate, while the glutamate acts as a catalytic base (Gorbalenya & Koonin, 1993). Motif II in RecQ proteins (DExHC) is canonical (Morozov *et al*., 1997) and is therefore likely to carry out the typical chemical functions of Walker B motifs (Figure 3b). Other residues outside of motifs I and II will likely contribute to ATP binding and hydrolysis in RecQ proteins, including an arginine residue in motif VI (R329 in *E. coli* RecQ) that is near the triphosphate moiety in the ATPγS-bound structure of the *E. coli* RecQ catalytic core (Bernstein *et al*., 2003). This residue could potentially stabilize an ATP hydrolysis transition-state intermediate.

Mutational analyses have shown that motif I is critical for RecQ function *in vivo* and *in vitro*. In all cases studied to date, mutation of the phosphate-binding lysine residue in RecQ motif I leads to a loss of helicase activity and deleterious effects *in vivo*. In human WRN, this mutation produces a variant that lacks helicase activity *in vitro* (Gray *et al*., 1997) and that when transgenically introduced into mice acts in a dominant negative fashion, inducing a Werner's syndrome phenotype in tail-derived fibroblasts (Wang *et al.*, 2000). Mutations of the equivalent lysine residue in Sgs1 results in loss of helicase function *in vitro* (Lu *et al.*, 1996) and a failure to complement the *sgs1* phenotype of hypersensitivity to several DNA-damaging agents *in vivo* (Mullen *et al.*, 2000; Onoda *et al*., 2000; Saffi *et al.*, 2000). Interestingly, these Sgs1 variants still complement aberrant growth rate phenotypes that arise from deletion of both *SGS1* and genes encoding either topoisomerase I or III proteins, implying that helicase activity is not needed for *in vivo* activity in some contexts (Lu *et al*., 1996; Mullen *et al*., 2000). Thus, it appears that ATPase and DNA helicase activities are important for most, but not all, RecQ cellular functions.

Roles of Other Elements in the RecQ Helicase Domain. Other regions in the RecQ helicase domain are likely to be important for RecQ function, but few experiments have probed their roles. One obvious role that the helicase domain must serve is DNA binding. In other helicases, DNA-binding surfaces are composed primarily of motifs Ia, IV, and V (Caruthers & McKay, 2002), and it is possible that that same is true in RecQ proteins as well. Mutations in several of these motifs in Sgs1 result in variants that do not complement *sgs1* DNA-damage sensitivity and hyperrecombination phenotypes (Ui *et al.*, 2001). Another potential site for DNA binding was revealed by the structure of the *E. coli* RecQ catalytic core. In RecQ family members, motif II is always directly followed by an aromatic-rich sequence ($SxWGx_2FRx_2Y$). The structure showed that this peptide maps to a very similar location on the surface of the helicase domain as an aromatic-rich sequence in PcrA helicase that forms a major binding site for ssDNA (Velankar *et al*., 1999), although the PcrA motif follows motif III in the protein. Alteration of either the tryptophan or arginine from this motif in Sgs1 leads to DNA-damage sensitivity and hyperrecombination phenotypes associated with *sgs1* mutations (Ui *et al*., 2001), consistent with an important role for this motif in Sgs1 protein function.

RecQ C-Terminal (RecQ-Ct) Domain of RecQ Proteins

Most RecQ family members have a second conserved region C-terminal to their helicase domains called a RecQ-Ct domain (Morozov *et al*., 1997; Figure 1). This region is conserved in all known bacterial RecQ homologs, as well as in human RecQ1 (Puranam & Blackshear 1994; Seki *et al.*, 1994), BLM (Ellis *et al*., 1995), WRN (Yu *et al*.,

1996), and RecQ5β (Sekelsky *et al.*, 1999; Shimamoto *et al.*, 2000). A RecQ-Ct domain is similarly conserved in known yeast RecQ proteins Sgs1 (Gangloff *et al.*, 1994; Lu *et al.*, 1996; Watt *et al.*, 1995) and Rqh1 (Murray *et al.*, 1997)), as well as in other eukaryotic RecQ proteins. RecQ-Ct domains are lacking, however, in human RecQ4 (Kitao *et al.*, 1998) and two of the protein products of alternatively spliced isoforms of the human *RECQ5* gene (RecQ5α and Rec5 γ; Sekelsky *et al.*, 1999; Shimamoto *et al.*, 2000), although a RecQ-Ct domain is found in the *D. melanogaster* RecQ5α protein (Sekelsky *et al.*, 1999).

The structure of the RecQ-Ct domain in *E. coli* RecQ reveals that it is made up of two elements: a platform of four α-helices that ligand a Zn^{2+} ion via four highly conserved cysteine side chains, and a more poorly conserved winged-helix (WH) subdomain that shares structural similarity with several DNA-binding proteins (Bernstein *et al.*, 2003; Figures 3c and 3d). Here we will review cellular, biochemical, and structural aspects of the two subdomains that form the RecQ-Ct domain. It is worth noting that existing literature often defines the RecQ-Ct domain by only the well-conserved Zn^{2+}-binding region, but in this review we will consider the more poorly conserved WH region as a part of the RecQ-Ct domain since it is structurally continuous with the Zn^{2+}-binding region.

Zn^{2+}-Binding Platform in the RecQ-Ct Domain. In bacterial RecQ proteins, a motif that includes four consistently spaced cysteine residues is conserved near the RecQ-Ct domain N-terminus. The sequence of this region is C++x_{13-17}CxxCxxC, where "+" residues are frequently electropositive (52 or 53 of 70 bacterial sequences for the first or second "+," respectively). Similar cysteine arrays are also conserved in most eukaryotic RecQ proteins but differ slightly from those in bacteria (C++x_{16-22}(C/E/D)xxxCxxC in *S. cerevisiae* Sgs1, *S. pombe* Rqh1, *Xenopus laevis* FFA-1, and human RecQ1, WRN, BLM, and RecQ5β). Prior to solution of a RecQ-Ct domain structure, it was unclear how this region of the protein might fold. However, the region was clearly reminiscent of Zn^{2+}-binding domains. In particular, the cysteine array is quite similar to a motif conserved in the bacterial DNA polymerase III holoenzyme γ, τ, and δ' subunits (Cx_{8-13}CxxCxxC). An X-ray crystal structure of the $\gamma/\tau/\delta'$ complex from *E. coli* showed that each of the subunits binds a Zn^{2+} ion via this motif (Jeruzalmi *et al.*, 2001), which lent credence to the idea that the RecQ-Ct domain could bind Zn^{2+} as well. Zn^{2+} also has an inhibitory effect on the DNA-unwinding activity of *S. cerevisiae* Sgs1 (Bennett *et al.*, 1998), raising the possibility that a regulatory Zn^{2+}-binding site exists in RecQ proteins.

The RecQ-Ct domain of *E. coli* RecQ was shown to form a Zn^{2+}-binding site by solution of the structure of its catalytic core (Bernstein *et al.*, 2003; Figure 3c). The domain binds Zn^{2+} using the four conserved cysteines of the RecQ-Ct cysteine motif, which are brought together to form the metal binding site by packing four α-helices together in a flat arrangement. Two of the cysteines lie at the N-termini of helices, making the helical dipole moment a likely contributor to their ionization (Kortemme & Creighton, 1995) and, therefore, to efficient Zn^{2+} binding. Additional sequence characteristics within the cysteine array could affect the pK_a values of the cysteine side chains. The first of these is the C++ motif. The electropositive cluster that follows the cysteine could act to lower its pK_a, making it a better ligand for Zn^{2+}. Interestingly, this feature might also make the cysteine more prone to oxidation, as has been seen in similar cysteine motifs of redox-reactive transcription factors (*e.g.*, NFI (Bandyopadhyay & Gronostajski, 1994), NF-κB (Matthews *et al.*, 1992), OxyR (Storz *et al.*, 1990), AP-1 (Abate *et al.*, 1990), and E2 (McBride *et al.*, 1992)). A second feature that could contribute to Zn^{2+}-binding site cysteine ionization is that three of the cysteines are in CxxC motifs. The pK_a values of N-terminal cysteines in CxxC motifs have been shown to depend in part on the identity of the two residues linking the cysteines (Chivers *et al.*, 1997). This pK_a dependence reflects a redox potential that can vary from strongly reducing to oxidizing, and could be different for various RecQ proteins. The redox chemistry of cysteine residues in the RecQ Zn^{2+}-binding site is of interest because it will ultimately be related to the enzyme's affinity for the metal in diverse redox conditions, and thus could represent a mechanism for regulating cellular RecQ function.

Several studies of RecQ variants in which conserved cysteine motif residues have been altered indicate the importance of this region to enzyme function. Two of the known *BLM* clinical missense mutations that lead to Bloom's syndrome alter codons for cysteines in the RecQ-Ct region (Ellis *et al.*, 1995; Foucault *et al.*, 1997). These mutations (C1036F, from the C++ motif, and C1055S, the first cysteine in the C/E/DxxxCxxC motif) change residues analogous to two that bind Zn^{2+} directly in *E. coli* RecQ. The human C1055S BLM variant is inactive as an ATPase and helicase *in vitro*, and it fails to reduce the hyperrecombination phenotype of BLM cells in transfection experiments (Neff *et al.*, 1999), indicating a severe defect in protein function. Analogous point mutations have been made as murine BLM variants, and both result in the loss of ATPase and helicase activity *in vitro* (Bahr *et al.*, 1998). Additionally, *S. cerevisiae SGS1* genes mutated at any of its conserved cysteines or the first arginine of the C++ fail to complement the DNA-damage sensitivity and hyperrecombination phenotypes of an *sgs1* strain (Onoda *et al.*, 2000; Ui *et al.*, 2001). These observations suggest that the Zn^{2+}-binding portion of the RecQ-Ct domain plays an important role in RecQ-catalyzed reactions.

What might the Zn^{2+}-binding region in RecQ proteins do? Zn^{2+}-binding domains in proteins have been found to be critical for a variety of functions, including activities in DNA binding, as surfaces that mediate interactions with other proteins and as stabilizing elements for protein structure (Berg & Shi, 1996). Experimental support for all of these possibilities exists in the RecQ literature. The Zn^{2+}-binding module in the *E. coli* RecQ helicase structure is present on the surface of the protein where it could potentially bind to proteins or DNA (Bernstein *et al*., 2003). It also lies adjacent to the WH domain (described below), which binds DNA (von Kobbe *et al.*, 2003) and heterologous proteins in the WRN protein (Brosh *et al.*, 2001b; von Kobbe & Bohr, 2002). The Zn^{2+}-binding portion of the RecQ-Ct domain could potentially form extended DNA, protein binding sites, or both with the WH domain. In addition, mutation of residues that are analogous to the *E. coli* RecQ Zn^{2+}-binding cysteines in an otherwise well-folded BLM deletion protein results in variants that are rapidly degraded by proteases, which could indicate an impairment in folding and/or thermostability (Janscak *et al.*, 2003). Thus, the roles played by the Zn^{2+}-binding region in RecQ proteins appear to be quite complex.

Winged-Helix Subdomain of the RecQ-Ct Domain. A weakly conserved stretch of ~100 amino acids follows the Zn^{2+}-binding region of the RecQ-Ct domain in most RecQ proteins. While the sequence of this portion of the RecQ-Ct domain did not help to define its structure and function, several studies described below have demonstrated its direct involvement in DNA and protein binding. The structure of the *E. coli* RecQ catalytic core shows that this region forms a WH domain that is structurally homologous to a number of DNA-binding domains from other proteins (Bernstein *et al*., 2003; Figure 3d). WH domains are typically small (~100 residues) and are organized such that a prominent helix called the "recognition helix" is presented on the surface (Gajiwala & Burley, 2000). In most WH proteins studied to date, the recognition helix makes extensive major groove contacts with dsDNA. These contacts are often sequence-specific (*e.g.*, HNF-3γ; Clark *et al.*, 1993), although nonspecific canonical WH-DNA contacts have also been proposed (*e.g.*, histone H5; Ramakrishnan *et al.*, 1993). The wing of the WH fold, formed by β-strands on the same face of the domain as the recognition helix, can also make contacts with the DNA, generally with the minor groove. In addition to their roles in DNA binding, WH domains have proven important for mediating protein–protein contacts, including roles in homodimerization (heat-shock transcription factor; Littlefield & Nelson, 1999) and heterodimerization (E2F-DP2; Zheng, *et al.*, 1999). Interestingly, WH domains have also been proposed to play distinct roles in associating with HJ DNA (Zlatanova & van Holde, 1998), which are targets for several RecQ helicases (as described below). While the structural details of WH-mediated interactions in RecQ proteins are not yet well defined, biochemical evidence of its involvement in both structure-specific DNA binding and heterologous protein association are substantial.

RecQ variants that lack the WH portion of the RecQ-Ct domain are functionally deficient *in vitro* and *in vivo*. Two studies in *S. cerevisiae* have shown that deletions that remove either the entire RecQ-Ct domain (Ui *et al*., 2001) or just the WH region portion of Sgs1 (Mullen *et al*., 2000) along with the rest of its C-terminus lead to hyper-recombination phenotypes that are indistinguishable from *sgs1* strains. Both studies indicate that the Sgs1 deletion variants only partially complement the methyl methane sulfonate (MMS)-hypersensitivity phenotype of *sgs1* cells, further implying impaired activity for both proteins *in vivo*. Importantly, Sgs1 constructs that leave the WH domain intact but remove the other C-terminal elements still complement these *sgs1* phenotypes (Mullen *et al*., 2000, 2001), indicating that these phenomena are directly related to the WH domain. In addition, the activity of a BLM variant that contains only the helicase domain and the Zn^{2+}-binding portions of the RecQ-Ct domain, but not the WH domain, is significantly impaired for unwinding HJ DNA relative to a BLM variant that contains the WH domain (Janscak *et al*., 2003). Related to this point, domain mapping experiments with WRN protein have shown that its WH region binds a variety of DNA substrates (ss, ds, forked, HJ, and 5′ overhang-containing DNA), although the region appears to be dispensable for DNA unwinding (von Kobbe *et al*., 2003). In addition, Bohr and coworkers have demonstrated that the WH portion of WRN is responsible for interaction with the 5′ flap endonuclease protein (FEN-1) (Brosh *et al*., 2001b) and that it contains a signal that is critical for its subcellular localization to the nucleolus (von Kobbe & Bohr, 2002), thus implicating the domain in protein interactions as well. These insights, together with the RecQ WH domain structure, should aid in directing future studies that will dissect high-resolution details of RecQ's interaction surfaces.

Helicase and RNase D C-Terminal Domain of RecQ Proteins

The third region of homology in RecQ proteins is the HRDC domain (Morozov *et al*., 1997; Figure 1). This region shares sequence homology with a domain in the RNase D family of nucleases. Since the HRDC domain is present in two enzymes involved in nucleic acid metabolism, it has been proposed to be involved in nucleic acid binding, the major function RecQ and RNase D have in common (Morozov *et al*., 1997). The HRDC region is the most variable of the major conserved RecQ domains,

with several examples of RecQ enzymes lacking an HRDC domain altogether (*e.g.*, human RecQ1, RecQ4, and all RecQ5 splice variants) and other examples of bacterial RecQ enzymes with multiple tandem HRDC domains (two in *Rhodobacter sphaeroides* and three in *Deinococcus radiodurans*, *Neisseria meningitidis*, and *Neisseria gonorrohoeae* RecQ). How this structural heterogeneity affects the functions of different RecQ proteins is presently unclear.

Experiments have shown that the HRDC regions from various RecQ proteins can be expressed as independently folded domains that posses DNA binding activities *in vitro*. First, the NMR structure of the HRDC domain from Sgs1 has been determined and shown to comprise of 5 helices folded together in a compact module (Figure 3e; Liu *et al.*, 1999). This domain has furthermore been shown to bind to DNA (Liu *et al.*, 1999). Chemical shift experiments indicate that a positively charged surface of the HRDC domain interacts preferentially with ssDNA, verifying its role in DNA-binding. Structural comparisons show that the HRDC fold is conserved with auxiliary DNA binding folds in other proteins, including Rep (Korolev *et al.*, 1997) and PcrA (Subramanya *et al.*, 1996) helicases, DNA polymerase β (Liu *et al.*, 1996; Pelletier *et al.*, 1994), Xer recombinase (Subramanya *et al.*, 1997), and T7 RNA polymerase (Jeruzalmi & Steitz, 1998), again consistent with a DNA-binding role for the domain. Second, domain mapping in *E. coli* RecQ showed that its HRDC domain could be separated from the catalytic core of the protein (Bernstein & Keck, 2003). This domain also binds DNA *in vitro* (D. A. Bernstein & J. L. Keck, unpublished observation). Finally, the HRDC domain of human WRN protein has been purified and shown to bind to DNA (von Kobbe *et al.*, 2003). The WRN HRDC domain preferentially binds to HJ and forked DNA substrates, although binding appears to be higher affinity when sequence elements including the RecQ-Ct domain or others C-terminal to the HRDC domain are included with the domain variant.

Although the HRDC domain clearly plays a role in DNA binding, its overall importance to catalysis and *in vivo* function is poorly understood. Hypersensitivity of *sgs1 S. cerevisiae* strains to DNA-damaging agents can be complemented equally well by either full-length Sgs1 or a C-terminally truncated variants lacking the HRDC domain (Mullen *et al.*, 2000, 2001). Also, recombinant fragments of Sgs1, *E. coli* RecQ, and human BLM lacking their HRDC domains are active as ATPases and DNA helicases *in vitro* (Bennett *et al.*, 1998; Bernstein & Keck, 2003; Janscak *et al.*, 2003; Lu *et al.*, 1996), and human WRN protein variants that lack an HRDC domain still bind to DNA (von Kobbe *et al.*, 2003). These data imply that the HRDC domain is largely dispensable for RecQ activity. However, an Sgs1 variant lacking the HRDC domain does not complement the aberrant growth phenotypes in strains lacking both *SGS1* and genes encoding either topoisomerase I or III, whereas full-length Sgs1 does complement. This implies that the HRDC domain is required for some cellular functions involving topoisomerases (Lu *et al.*, 1996; Mullen *et al.*, 2000).

What role could the HRDC domain play in RecQ helicase cellular functions? In Sgs1 the HRDC domain has been shown to preferentially bind ssDNA (Liu *et al.*, 1999), while in contrast the HRDC domain from WRN preferentially binds HJ and forked DNA structures (von Kobbe *et al.*, 2003). Indirect experiments with human BLM deletion proteins are consistent with a role for its HRDC domain in ssDNA binding similar to that seen in Sgs1 (Janscak *et al.*, 2003). HRDC-directed binding specificity could potentially help target RecQ proteins to distinct DNA substrates in cells with multiple RecQ homologs (*e.g.*, humans), while providing a more general enhancement to DNA binding in other organisms with just one *recQ* gene (*e.g., S. cerevisiae* or *E. coli*). Moreover, the auxiliary DNA-binding function of the HRDC domain could alter the kinetics, processivity, or both of DNA unwinding in RecQ proteins (Bernstein & Keck, 2003). For example, RecQ proteins with multiple HRDC domains might unwind DNA more processively than related homologs with one or no HRDC domain. In addition, the HRDC domain could provide a surface for protein–protein interactions. This potential function could help explain why the domain is important in some cellular contexts (*i.e.*, in *S. cerevisiae* cells lacking topoisomerases I or III (Lu *et al.*, 1996; Mullen *et al.*, 2000)) but not others. Further biochemical and cell biological experiments are required to determine the precise roles played by this domain in RecQ protein function.

CELLULAR FUNCTIONS OF RecQ HELICASES

The studies outlined above indicate that a wide variety of DNA structures are substrates for RecQ helicase activity. A similarly diverse number of functions have been proposed for RecQ proteins, with the focus being on how these act to prevent excessive recombination from destabilizing the genome. Several of the proposed functions of RecQ proteins are at different steps in the replication pathway, and there is ample evidence for RecQ function in replication. For example, the BLM and Sgs1 protein levels peak during S-phase (Dutertre *et al.*, 2000; Frei & Gasser, 2000), and RecQ proteins interact with a number of factors involved in DNA replication, including DNA topoisomerases, proliferating cell nuclear antigen (PCNA), RPA, and DNA polymerase δ (Brosh *et al.*, 2000; Gangloff *et al.*, 1994; Lebel *et al.*, 1999; Watt *et al.*, 1995; Wu *et al.*, 2000). In addition, RecQ helicases colocalize with sites of DNA synthesis in yeast, xenopus, and humans (Chen *et al.*, 2001; Cobb *et al.*, 2003; Davies *et al.*, 2004; Frei & Gasser, 2000; Rassool, 2003; Sengupta *et al.*, 2003), and cells derived

from Bloom's syndrome and Werner's syndrome patients exhibit abnormal replication intermediates (Lonn *et al.*, 1990; Poot *et al.*, 1992). We will first discuss how RecQ helicases act to prevent the breakdown of replication forks, and, secondly, how RecQ proteins also act following the breakdown of replication forks to prevent chromosome crossing-over.

Maintenance of Replication Fork and Checkpoint Activity

One role of RecQ helicases is to function as part of the S-phase checkpoint, a surveillance mechanism for checking the accurate replication of DNA and the removal of DNA damage. In *S. cerevisiae*, two S-phase checkpoint pathways have been identified, the intra-S checkpoint and the replication checkpoint. Sgs1 has been implicated in both pathways, although its major role appears to be in the intra-S checkpoint pathway, required for response to DNA-damaging agents such as MMS, reviewed recently in Kolodner *et al.* (2002). Treatment with hydroxyurea (HU), which depletes cells of nucleotides, also activates the intra-S checkpoint, leading to slowing of replication fork movement, down-regulation of late-firing origins, and arrest of spindle elongation. The intra-S checkpoint is composed of two branches, one that requires Rad17, Rad24 and Mec3, and the other that requires Sgs1. Inactivation of either branch has only a small effect on genome stability, but inactivation of both branches results in a synergistic increase in genome instability (Frei & Gasser, 2000; Myung & Kolodner, 2002). This can lead to gross chromosome abnormalities, such as deletion of parts of chromosome arms and the *de novo* addition of a telomere to the broken end (Myung & Kolodner, 2002). An even larger increase in genome instability is observed if both the intra-S checkpoint branches and the replication checkpoint pathway are simultaneously inactivated, resulting in a 12,000-fold increase in genome rearrangement (Myung *et al.*, 2001b).

The role of Sgs1 in the *S. cerevisiae* intra-S checkpoint is thought to be that of a detector of DNA damage, perhaps acting to recognize stalled forks. Sgs1 signals the activation of Rad53, probably via a direct effect, since Sgs1 colocalizes with Rad53 and is required for its redistribution during S-phase, which in turn induces most of the downstream events (Frei & Gasser, 2000). In *S. pombe*, the RecQ homolog, Rqh1, has also been implicated in the response to DNA damage. In this case, however, there is no clear checkpoint defect in *rqh1*$^-$ cells treated with HU, but after release from HU arrest these cells show a defect in chromosome segregation and a characteristic "cut" phenotype (Stewart *et al.*, 1997). In contrast, *rqh1*$^-$ cells exhibit a checkpoint defect in response to MMS, indicating that the nature of the DNA damage can determine the checkpoint response (Marchetti *et al.*, 2002).

Sgs1, BLM, and WRN proteins all localize to sites of DNA synthesis during replication, particularly so following DNA damage or the arrest of replication forks, suggesting that RecQ function in replication is conserved (Constantinou *et al.*, 2000; Franchitto *et al.*, 2003; Frei & Gasser, 2000). Furthermore, the BLM protein has been found to physically associate with two checkpoint kinases; ATM kinase (ataxia telangiectasia mutated, a homolog of *S. cerevisiae* Tel1) and ATR kinase (ataxia telangiectasia, a homolog of *S. cerevisiae* Mec1; Beamish *et al.*, 2002; Davies *et al.*, 2004). ATM has also been shown to phosphorylate BLM in response to ionizing radiation (Beamish *et al.*, 2002) while ATR phosphorylated BLM following replicative stress (*e.g.*, HU treatment; Davies *et al.*, 2004). In fact, BS cells expressing a mutant form of the BLM protein that lack the sites for phosphorylation by ATR are unable to recover from HU-induced replication arrest. Instead, following removal of HU, the cells arrest in G2, reminiscent of the defect seen in *S. pombe rqh1*$^-$ mutants following HU treatment and release (Davies *et al.*, 2004). The authors suggest that the presence of the mutant BLM protein causes unresolved recombination intermediates to linger in the HU-treated cells, preventing recovery from S-phase arrest.

Independent of checkpoint function, RecQ helicases may also aid replication by unwinding secondary structures preceding a replication fork that might otherwise impede fork progression (see Figure 4). If these "roadblocks" to replication are not removed the replication fork could stall, leading to genomic instability as the cell tries to restart the stalled fork by homologous recombination. One potential roadblock is G4 DNA because this is a stable structure likely to block translocating protein complexes and is also an efficient substrate for RecQ helicases. Indeed, the WRN protein has been shown to aid DNA polymerase δ in replicating through G4 and hairpin structures (Kamath-Loeb *et al.*, 2001). This result is consistent with the slower elongation rate of polymerases in human WS and BS cells (Lonn *et al.*, 1990; Poot *et al.*, 1992).

In contrast to WS and BS cells, *sgs1*Δ cells were recently shown to progress through S-phase faster than wild-type cells as a result of faster polymerase elongation rates. Sgs1 affects the rate of fork progression independently of its checkpoint function, as *rad53*Δ cells showed normal replication rates in undamaged cells (Versini *et al.*, 2003). This surprising result may be due to the fact that the *S. cerevisiae* genome is AT rich compared to the GC-rich genomes of mammalian cells, which are therefore more likely to form stable secondary structures such as G4 DNA. Significantly, although overall replication proceeds faster in *sgs1*Δ cells, the completion of replication at the ribosomal DNA (rDNA) locus is impaired. The rDNA contains a high density of replication fork barriers and is therefore more likely to be prone to replication fork collapse.

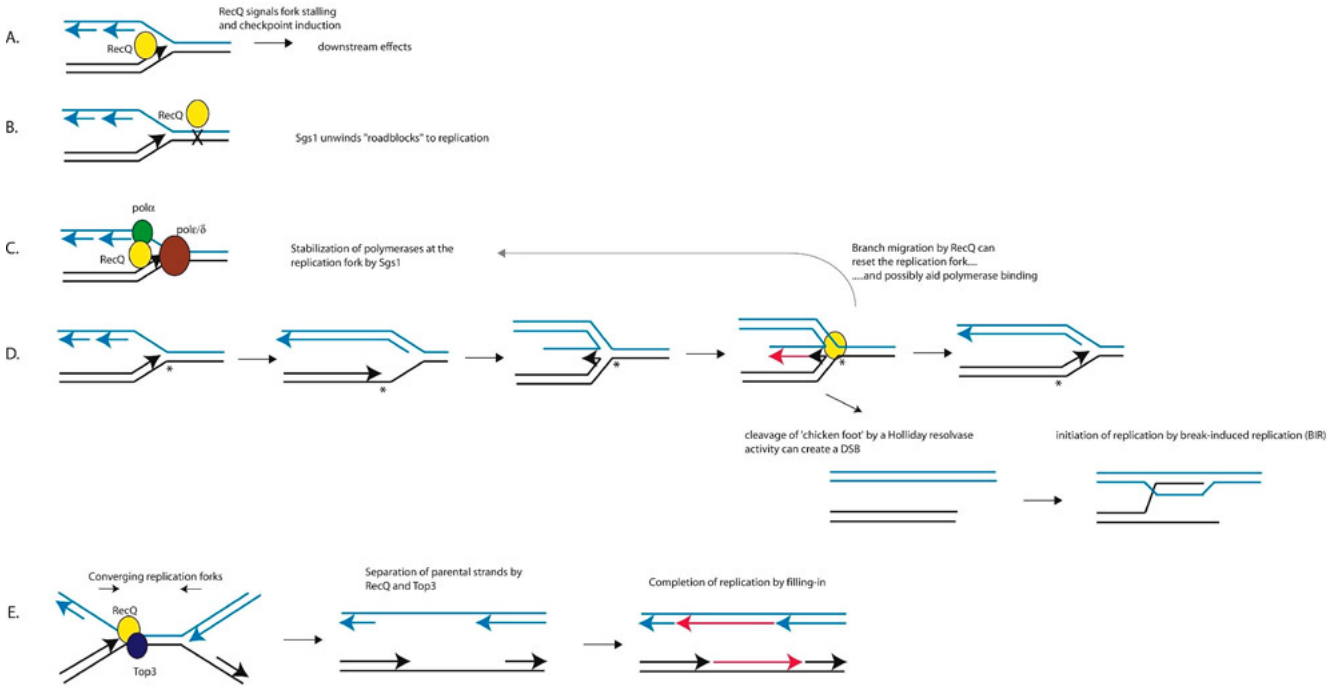

FIG. 4. Mechanisms by which RecQ helicases act to maintain or regulate the replication fork. RecQ helicases have been implicated in helping to prevent the breakdown of replication by several mechanisms. (a) RecQ helicases can signal to the checkpoint response the arrest of a replication fork. (b) Potential roadblocks ahead of the advancing fork can be unwound by RecQ. (c) Stabilization of polymerases at the replication fork by RecQ. (d) A block of leading strand synthesis (asterisk) can lead to the formation of an HJ intermediate. RecQ helicases can promote reverse branch migration of the junction and potentially reset the replication fork. (e) RecQ helicases may allow the completion of replication by acting with DNA topoisomerases (*e.g.*, Top3) to unravel the duplex DNA between converging replication forks. Red lines indicate new DNA synthesis.

Versini *et al.* (2003) found that the structure of the rDNA array was altered during replication in *sgs1*Δ cells, and that the altered mobility could be suppressed by inactivation of homologous recombination in a *rad52*Δ mutant. The presence of Sgs1 is therefore especially important at sites of stalled replication, and in its absence, rDNA instability results from increased recombination. In mammalian cells, replication forks may stall more frequently throughout the genome due to the higher frequency of G4 structures or the presence of higher order chromatin structures, accounting for the slower replication rates in BS and WS cells.

Can RecQ helicases act directly to stabilize stalled replication forks? The efficient recovery of cells from replication arrest requires the stabilization of replication factors at stalled forks, allowing replication to quickly resume once the genotoxic stress has been removed (Desany *et al.*, 1998). Recent evidence suggests that Sgs1 plays a direct role in the stable binding of polymerases α and ε at stalled replication forks. In the absence of Sgs1, the level of these DNA polymerases at replication origins is reduced 2- to 4-fold (Cobb *et al.*, 2003). The helicase activity of Sgs1 is required, but the effect is not dependent on Rad53, suggesting that polymerase stabilization was independent of the intra-S checkpoint pathway (Cobb *et al.*, 2003). How can a helicase stabilize polymerase binding to a stalled fork? As shown in Figure 4, stalling of the replication fork at a DNA lesion in the leading strand can lead to regression of the fork and the formation of a 4-way junction or "chicken-foot" structure. Replication past the lesion can occur by the leading strand copying the intact DNA sequence from the lagging strand. Resetting of the replication fork by RecQ-mediated unwinding of the 4-way junction results in the leading strand bypassing the DNA lesion and the resumption of normal DNA synthesis. It has been suggest that RecQ-catalyzed reverse branch migration of the 4-way junction could be required for polymerase stabilization, perhaps by maintaining a normal replication fork structure (Cobb *et al.*, 2003). In the absence of RecQ helicases, cleavage of the chicken-foot intermediate by a Holliday resolvase would generate a double-strand break (DSB) and initiate homologous recombination (Figure 4). This has been shown to occur in *E. coli*, where stalling of replication forks leads to RecA-mediated recombination events (Michel, 2000; Michel *et al.*, 2001). Thus, fork reversal may be important for preventing excessive recombination following stalling of replication forks. In fact, cleavage of

the chicken-foot structure and subsequent recombinational exchange in BS cells has been proposed to account for the high level of sister-chromatid exchanges (SCEs) observed in these cells.

One further mechanism has been proposed to account for the role of RecQ helicases in promoting replication, and that involves solving the problem of converging replication forks. As replication forks converge, unraveling of the parental duplex ahead of the forks becomes more difficult, and this could inhibit movement of the forks relative to one another (Wang, 1991). This topological barrier could be removed by the concerted action of a helicase and a DNA topoisomerase (Gangloff *et al*., 1994; Wang, 1991). The helicase unwinds the intervening duplex between the replication forks, and the DNA topoisomerase decatenates the parental strands of the template (see Figure 4). This model is consistent with a number of genetic and biochemical observations linking RecQ proteins with DNA topoisomerases, particularly DNA topoisomerase III (Top3). For example, deletion of the RecQ homolog in *S. cerevisiae* and *S. pombe* suppresses the hyperrecombination and cell growth defects of *top3* mutants (Gangloff *et al*., 1994; Goodwin *et al.*, 1999; Maftahi *et al.*, 1999). RecQ proteins and DNA topoisomerase III homologs have also been shown to directly interact in yeast and humans (Bennett & Wang, 2001; Fricke *et al.*, 2001; Gangloff *et al*., 1994; Johnson *et al.*, 2000; Shimamoto *et al*., 2000; Wu *et al*., 2000). Furthermore, the Top3 interaction domain of BLM is required to prevent elevated SCE (Hu *et al.*, 2001). It should be noted, however, that alternative functions have been proposed to account for the conserved role of the RecQ/Top3 complex, as discussed below.

Action of RecQ Helicases Following Breakdown of the Replication Fork

Mutations in *recQ* genes do not generally affect vegetative growth, although RecQ function is essential for viability in certain mutant backgrounds. In *S. cerevisiae*, *SGS1* exhibits synthetic lethal phenotypes with a number of genes, including the *SRS2* gene, which also encodes a helicase with 3′ to 5′ activity. Both *sgs1* and *srs2* mutants are viable, yet the *sgs1 srs2* double mutant exhibits an extreme growth defect. This was initially linked to an essential role of these helicases in unwinding DNA during replication fork movement (Lee *et al.*, 1999). However, the growth defect is suppressed by mutations in genes that act during the early stages of homologous recombination, including *rad51*, *rad52*, *rad55*, and *rad57* (Fabre *et al.*, 2002; Gangloff *et al.*, 2000). This suggests that the Sgs1 and Srs2 proteins are required for processing intermediates in homologous recombination; in their absence, these recombination intermediates are lethal to the cell.

Present models of RecQ function suggest that the toxic recombination intermediates are formed following breakdown of the replication fork and the subsequent initiation of recombination (see Figure 5). In *S. cerevisiae*, these recombination events are initiated from ssDNA structures rather than DSBs, since Rad52 is required for the processing of DSBs yet mutants in *rad52* suppress the growth defect of *sgs1 srs2* cells (Fabre *et al*., 2002). Figure 5 shows how a lesion blocking the synthesis of the lagging strand can generate a ssDNA gap, leading to strand invasion of the intact duplex. Depending on how this intermediate is processed, the outcome can produce gene conversion either with or without the concomitant crossing over of flanking chromosome arms, as outlined below.

In addition to *SRS2*, synthetic lethal interactions between *SGS1* and a number of other genes have been observed, including the *MUS81* and *MMS4* genes (Mullen *et al*., 2001; Ooi *et al.*, 2003). Once again, deletion of homologous recombination genes rescued the synthetic lethality of *sgs1 mus81* or *sgs1 mms4* strains, indicating that Mus81 and Mms4 are part of an alternative pathway to Sgs1 for the processing of recombination intermediates (Fabre *et al*., 2002). The Mus81 and Mms4 proteins form a heterodimer complex that acts as a structure-specific endonuclease, and homologs of these genes have been identified in other eukaryotes, including humans (Boddy *et al.*, 2001; Chen *et al*., 2001). Similar to the case in *S. cerevisiae*, *S. pombe* cells lacking either Rqh1 or Mus81 are viable, while the *rqh1*$^{-}$ *mus81*$^{-}$ double mutant is inviable (Boddy *et al.*, 2000). The complex isolated from *S. pombe* (known as Mus81/Eme1) has been shown to cleave HJs and forked DNA structures *in vitro* (Boddy *et al*., 2001; Chen *et al*., 2001). However, a recombinant form of the *S. pombe* Mus81/Eme1 complex purified from *E. coli*, as well as *S. cerevisiae* Mus81/Mms4 and the human Mus81-associated endonuclease, preferentially cleaves forked DNA structures rather than HJs (Constantinou *et al.*, 2002; Doe *et al.*, 2000; Kaliraman *et al.*, 2001). It is therefore unclear at present whether the true substrate for Mus81/Mms4 endonuclease is the HJ, the replication fork, or perhaps an intermediate form between the two (Gaillard *et al.*, 2003; Haber & Heyer 2001; Osman *et al.*, 2003).

A further link between RecQ helicases and HJ resolvases comes from the observation that defects in *S. pombe rqh1*$^{-}$ mutants and in human WS cells can be suppressed by expression of a bacterial HJ resolvase (Doe *et al*., 2000; Saintigny *et al.*, 2002). HJ-containing intermediates must therefore accumulate in cells lacking a functional RecQ activity. How can RecQ helicases function both to remove HJs and yet prevent chromosome crossing over? A recent report by Wu and Hickson suggests that RecQ helicases act in concert with DNA topoisomerase III to "dissolve" HJs while suppressing the formation of crossover products

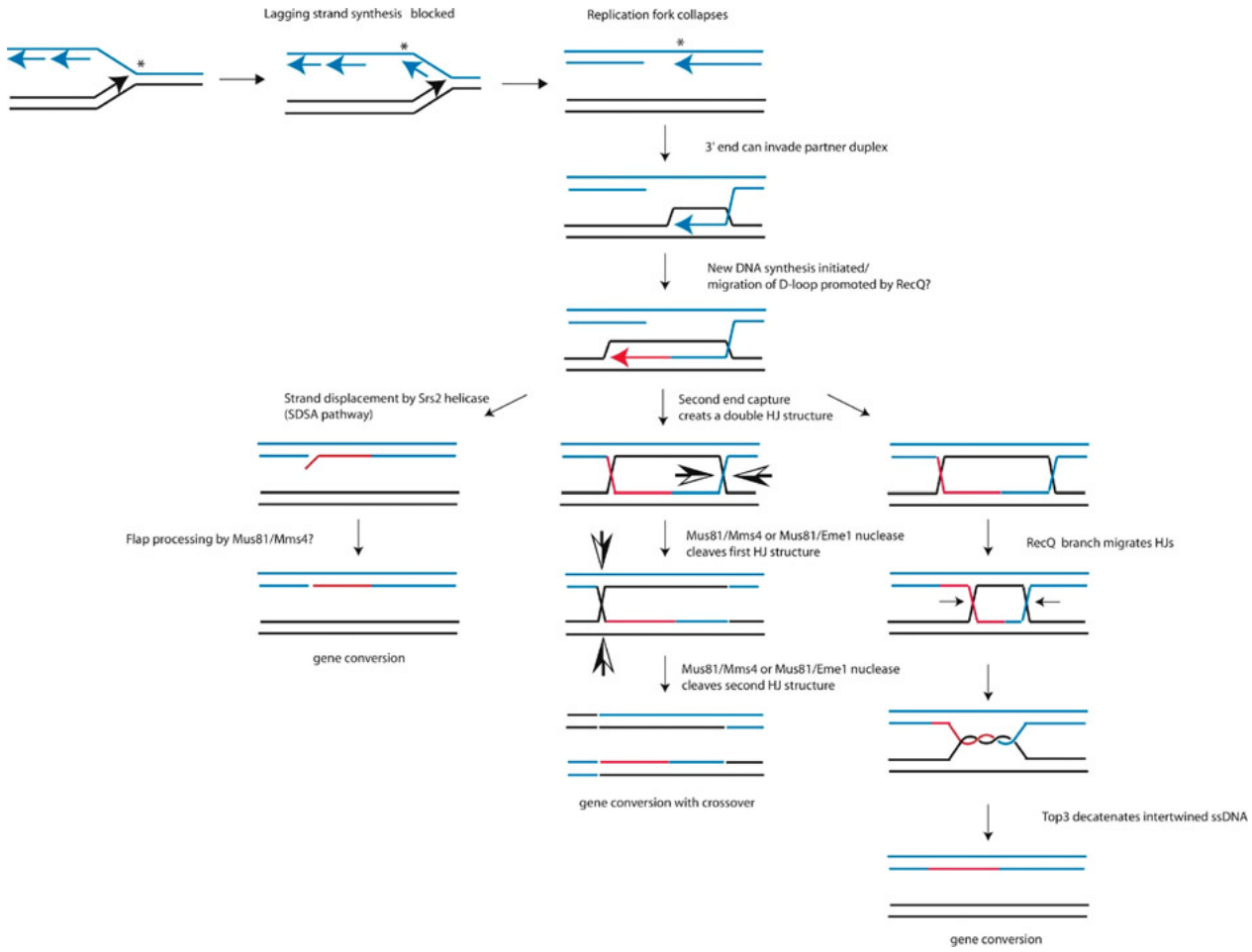

FIG. 5. Mechanisms by which RecQ helicases act following the breakdown of the replication fork. Replication fork breakdown is shown following a block in lagging strand synthesis (asterisk). The 3′ end at the ssDNA gap can invade the opposite duplex generating a D-loop structure. DNA synthesis and branch migration of the D-loop may be promoted by RecQ helicases. The D-loop intermediate may be processed in three ways. Firstly, the invading strand can be displaced by the Srs2 helicase as part of the SDSA pathway, and any remaining 3′ flap is removed by the Mus81/Mms4 nuclease. Alternatively, the invading strand can switch templates and generate a dHJ structure. This can be processed by a HJ resolvase activity, possibly Mus81/Mms4, to generate gene conversion products either with, or without, an associated crossover event (depending on the orientation of resolution of the HJs). Finally, the dHJ structure can be processed by RecQ helicases in association with DNA topoisomerase III. RecQ helicases unwind the DNA between the HJs, creating a substrate for decatenation by the topoisomerase. Both the SDSA pathway and the RecQ/Top3 pathway generate recombinants with no chromosome crossing over. New DNA synthesis is indicated by red lines.

(Wu & Hickson, 2003). Purified BLM protein was shown to act in concert with human DNA topoisomerase IIIα to process DNA structures containing double Holliday junctions (dHJs; see Figure 5). The BLM helicase acts to branch migrate and/or unwind the two HJs, generating a structure linked only by intertwined ssDNA, which is an efficient substrate for decatenation by type IA DNA topoisomerases. This model demonstrates how dHJ structures can be processed to generate only noncrossover products (Figure 5). dHJ resolution by a RecQ/Top3 complex had previously been proposed by several authors to account for the conserved interaction between RecQ helicase and DNA topoisomerase III (Gangloff *et al.*, 1999; Ira *et al.*, 2003; Kwan *et al.*, 2003).

The model presented in Figure 5 indicates three ways in which strand invasion intermediates can be processed in *S. cerevisiae*. Firstly, the Srs2 helicase can act as part of the synthesis-dependent strand-annealing (SDSA) pathway to promote gene conversion without the crossing over of chromosome arms. Srs2 may act to directly displace the invading strand from the donor duplex, or act to displace the strand-exchange catalyst Rad51, thereby preventing

the formation of a dHJ intermediate (Ira *et al*., 2003; Krejci *et al.*, 2003; Veaute *et al.*, 2003). Secondly, dHJs could be resolved sequentially by a junction-specific endonuclease, possibly the Mus81-associated endonuclease. This pathway can result in the formation of crossover products, as shown in Figure 5. Note that Mus81/Mms4 has also been implicated in the SDSA pathway in *S. cerevisiae* for the processing of 3′ flaps following strand displacement (de los Santos *et al.*, 2001, 2003; see Figure 5). Finally, RecQ/Top3 complexes can act to remove dHJs without the formation of crossover products. This model is also consistent with recent studies on double-strand break repair in *S. cerevisiae*, in which noncrossover and crossover products were shown to arise by distinct pathways (Ira *et al*., 2003). Haber and coworkers showed that Srs2 and Sgs1/Top3 were involved in suppressing crossover formation in DSB repair, and that overexpression of either helicase could complement for deletion of the other.

Studies on *Drosophila melanogaster* BLM have also found a role for RecQ helicases in suppressing crossover formation in DSB repair, although in this case the protein was proposed to function directly in the SDSA pathway (Adams *et al.*, 2003). In the absence of *D. melanogaster* BLM, recombination intermediates were channeled into an error-prone pathway, creating large deletions. The authors suggest that DNA synthesis is not processive following strand invasion and that BLM may act to promote repeated rounds of strand invasion. Thus, by promoting the SDSA pathway, *D. melanogaster* BLM may be closer in function to *S. cerevisiae* Srs2 than its RecQ homolog, Sgs1 (Ira *et al*., 2003; Figure 5).

RecQ Helicases in DNA Repair and Telomere Stability

In addition to the multitude of tasks attributed to RecQ helicases in replication, there are several other roles that these proteins may perform in the cell. Nonhomologous end joining (NHEJ) is used as an alternative mechanism to homologous recombination in the repair of DSBs and involves exonucleolytic processing of the ends of the break, followed by ligation between ends of limited homology (reviewed recently in Lees-Miller & Meek, 2003). BLM associates with proteins known to function in NHEJ, and loss of BLM results in increased NHEJ activity (Gaymes *et al.*, 2002; Yang, 2003). WRN has also been linked with NHEJ through its interactions with both the Ku heterodimer and DNA-dependent protein kinase, two components of the NHEJ machinery (Cooper *et al.*, 2000; Li & Comai, 2000; Li *et al.*, 2004; Yannone *et al.*, 2001). It has been suggested that these interactions regulate the exonuclease activity of WRN, limiting DNA degradation in NHEJ and preventing excessive DNA deletions (Bachrati & Hickson, 2003; Karmakar *et al.*, 2002; Yannone *et al*., 2001). Overexpression of Ku can also suppress some of the defects associated with mutations in the *D. melanogaster* BLM protein (Kusano *et al.*, 2001).

The WRN protein has also been recently linked to base excision repair (BER) since it has been found to interact with a DNA polymerase involved in BER, DNA polymerase β (Harrigan *et al.*, 2003). The exonuclease activity of WRN may act as a proofreading activity for this polymerase, since it lacks its own exonuclease function. The possible roles of WRN in BER have recently been reviewed (Harrigan & Bohr, 2003).

Several RecQ proteins have been shown to interact directly with mismatch repair proteins. The BLM protein interacts with MLH1 (Langland *et al.*, 2001; Pedrazzi *et al.*, 2001) and MLH6 (Pedrazzi *et al.*, 2003). The Sgs1-Top3 complex has also been implicated in interactions with mismatch repair proteins (Wang and Kung, 2002). Since mismatch proteins also act to limit recombination between similar, but not identical, DNA sequences, it is possible that RecQ proteins also act to limit recombination by reversing inappropriate heteroduplex intermediates. In favor of this, *sgs1* mutants show increased rates of recombination between homeologous (nearly identical) sequences (Myung *et al.*, 2001a).

It should also be noted that some of the functions of RecQ proteins in the S-phase may also be required in other phases of the cell cycle. Evidence from *S. pombe* indicates that Rqh1 functions with Top3 to repair UV-damaged DNA in G_2 (Laursen *et al.*, 2003) and ionizing radiation-induced DSBs in G_2 cells were shown to generate Rqh1 and Top3 foci (Caspari *et al.*, 2002). Again, Rqh1 was implicated to function both upstream and downstream of the recombination machinery.

Finally, a role for RecQ proteins in maintenance of telomeres has been reported. Telomeres are protective caps at the end of chromosomes, and in their absence chromosomes generate deletions and end-to-end fusions. They are typically G-rich, so that G4 structures could form that are substrates for unwinding by RecQ helicases. In addition, a role for Sgs1 has been identified in a telomerase-independent mechanism for extending telomeres. The process known as ALT (alternative lengthening of telomeres) is dependent on homologous recombination and requires the Sgs1 protein (Cohen and Sinclair, 2001; Huang *et al.*, 2001; Johnson *et al.*, 2001). The function of RecQ helicases in telomere maintenance might be conserved, as both BLM and WRN proteins have been shown to interact with the TRF2 telomere-binding protein (Opresko *et al.*, 2002).

SUMMARY

RecQ family DNA helicases have proven to be critical molecules for linking the processes of DNA replication, recombination, and repair in bacteria and eukaryotes. In

this review, we have summarized recent key findings that have helped define the many functions and mechanisms RecQ family members. The biochemical underpinnings of RecQ function are now being linked with their cellular roles. In addition, three-dimensional structures of RecQ protein domains are now available and should help direct future biochemical and genetic experiments. We have no doubts that more surprises remain to be uncovered in the RecQ family of enzymes.

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