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Review

Probing the structural basis of RecQ helicase function

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ABSTRACT

RecQ helicases are a ubiquitous family of DNA unwinding enzymes required to preserve genome integrity, thus preventing premature aging and cancer formation. The five human representatives of this family play non-redundant roles in the suppression of genome instability using a combination of enzymatic activities that specifically characterize each member of the family. These enzymes are in fact not only able to catalyze the transient opening of DNA duplexes, as any other conventional helicase, but can also promote annealing of complementary strands, branch migration of Holliday junctions and, in some cases, excision of ssDNA tails. Remarkably, the balance between these different activities seems to be regulated by protein oligomerization. This review illustrates the recent progress made in the definition of the structural determinants that control the different enzymatic activities of RecQ helicases and speculates on the possible mechanisms that RecQ proteins might use to promote their multiple functions.

Contents

1. Introduction

Approximately 1% of the open reading frames in the human genome encode for proteins that function as DNA or RNA helicases. These enzymes operate in all aspects of nucleic acid metabolism where the complementary strands of DNA:DNA, DNA:RNA, or RNA:RNA duplexes require to be opened. They can be divided into two classes on the basis of their translocation directionality that can be either 3′-5′ or 5′-3′. The reaction of translocation and transient separation of the complementary strands catalyzed by helicases is coupled to the binding and hydrolysis of nucleotide triphosphate (NTP) which provides the "fuel" for the helicase motor [1–3]. A detailed description of the different Superfamilies of helicases and their properties is outside the scope of this article, and we refer readers to recent reviews [3–5].

RecQ helicases are a sub-family of helicases that play an essential role in the maintenance of genome stability by acting at the interface between DNA replication, recombination and repair [6–9]. They derive their name from the prototypical member of the family discovered in *Escherichia coli* over 20 years ago [10]. Mutations in the genes encoding three of the five human RecQ homologs are linked to defined genetic disorders associated with genomic instability, cancer predisposition, and features of premature aging; namely, Bloom's

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Table 1
RecQ helicase members for which structural data are available to date. In parenthesis are indicated the amino acid residues in the respective domains that have been studied by X-ray crystallography or NMR.

RecQ helicase	RecQ domain structurally studied			References
E. coli RecQ		Helicase + RQC domain (1–516)	HRDC domain (524–609)	[42,65]
S. cerevisiae Sgs1			HRDC domain (1271–1351)	[68]
D. radiodurans DrRecQ			HRDC domain (751–824)	[66]
Human RECQ1		Helicase + RQC domain (63–592)		[43]
Human WRN	Exonuclease domain (1–333)	RQC domain (949–1092)	HRDC domain (1142–1242)	[53,54,67,73]

syndrome (*BLM*-defect), Werner's syndrome (*WRN*-defect), and Rothmund-Thomson, RAPADILINO and Baller-Gerold syndromes (all caused by *RECQ4*-defects) [11–15]. The different clinical features of these disorders support the notion that these human helicases have distinct functions in cells. No heritable cancer predisposition disorder has yet been associated to mutations in the remaining two human RecQ helicase genes, *RECQ1* and *RECQ5*. However, recent studies have linked a single nucleotide polymorphism present in the *RECQ1* gene to a reduced survival in pancreatic cancer patients [16].

Two important features distinguish RecQ helicases from the other helicases. First is their ability to unwind a variety of DNA structures in addition to standard B-form DNA duplexes. Biochemical studies have demonstrated that RecQ helicases unwind DNA with a 3′ to 5′ polarity and, although with some differences, are capable of unwinding a variety of DNA structures including forked duplexes, displacement loops (D-loops; an intermediate in homologous recombination reactions), triple helices, 3- or 4-way junctions, and G-quadruplex DNA [17–21]. Second is the capacity of RecQ helicases to catalyze multiple enzymatic activities in addition to DNA unwinding; they can promote branch migration of Holliday junctions for several kilobases [22,23], annealing of complementary single stranded DNA molecules [24–28], and, in some cases, nucleotide excision with a 3′ to 5′ polarity [29,30]. Consistent with their ability to unwind various DNA structures and promote multiple enzymatic activities, several cellular functions have been attributed to RecQ proteins, including roles in stabilization and repair of damaged DNA replication forks, telomere maintenance, homologous recombination, base excision repair, and DNA damage checkpoint signaling [6–8,31,32]. Moreover, recent studies pointed to important and distinct roles of the human RECQ4 and RECQ1 helicases in DNA replication initiation [33]. Thus, understanding how these enzymes function and regulate their various DNA processing activities is important to untangle the mechanisms that control the integrity of our genome, and prevent cancer and aging.

In this review, we provide an overview of the structural information available for the distinct domains responsible for the translocation, unwinding, strand annealing, branch migration, and exonuclease activities of RecQ helicases (Table 1 and Fig. 1). Interestingly, many of these activities seem to be regulated by protein oligomerization [34–37]. The function of the different assembly states of RecQ helicases is still the subject of debate. On the basis of the results obtained for some representatives of the family so far, it is tempting to speculate that RecQ helicases might share a common mechanism whereby smaller oligomers, are required for DNA unwinding, while higher-order oligomers are required for more specialized activities, such as Holliday junction branch migration/disruption and DNA strand annealing [37,38]. The current evidence that supports or contradicts this model is discussed. From a comparison of the RecQ helicase structures with the structures of other enzymes characterized by only one of the above mentioned activities, we speculate on the possible mechanisms by which RecQ helicases promote their multiple enzymatic activities.

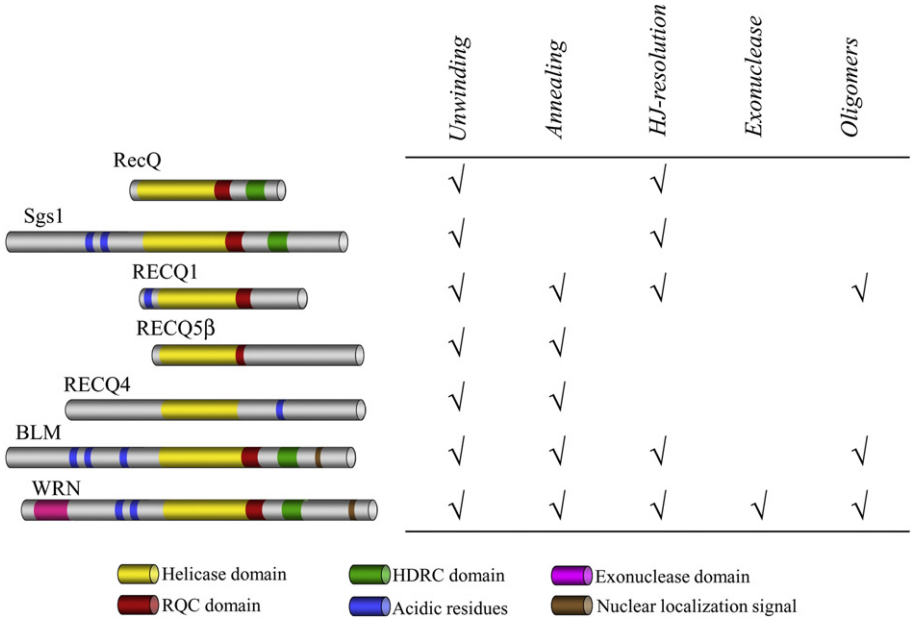

Fig. 1. The multiple enzymatic activities of RecQ helicases are regulated by their different domain organization and oligomeric states. (Left) Schematic representation of some of the best characterized members of the RecQ family color coded according to their structural domains: *E. coli* RecQ, *S. cerevisiae* Sgs1, and the five human RecQ helicases. Proteins are aligned according to the conserved helicase domain, which is shown in yellow. The conserved RQC and HRDC domains are shown in red and green, respectively. The exonuclease domain in the amino-terminal region of WRN is shown in pink. Regions containing patches of acidic residues are shown in blue. The nuclear localization signal sequences identified at the extreme carboxyl terminus of certain family members is shown as a brown bar. The remaining portions of each protein (gray) represent regions that are poorly conserved. The sizes of the individual domains are not to scale. At least three splice variants of the human RECQ5 protein are expressed, of which only the largest (β-isoform) is shown. (right) The different enzymatic activities reported for each helicase to date are indicated (unwinding, annealing, Holliday junctions (HJ) resolution, and exonuclease), along with their ability to form oligomers.

2. Structural determinants of RecQ helicases

RecQ helicases belong to the SF-2 superfamily [2,39]. There is a total of 29 members from different species identified so far, and this number is very likely to grow as more genomes will be sequenced; animal and plants genomes typically have 5–7 paralogues. As all bona fide helicases, the RecQ helicases contain a common core domain or "helicase domain" formed by two RecA-like domains, each one of a length of approximately 200 amino acids. This core domain provides ATP-dependent translocation along DNA or RNA molecules while true helicase activity often depends on additional structural elements. Located at the interface between the two RecA-like domains are the seven signature motifs of helicases, named motif I (or Walker A), Ia, II (or Walker B), III, IV, V and VI. Information available for other helicases of the SF-2 and SF-1 superfamilies indicates that these motifs are required for nucleotide binding and hydrolysis, and are also involved in ssDNA binding [3–5].

Multiple sequence alignment of SF-2 core helicase domains shows that RecQ helicases cluster in a specific branch, as they contain a number of sequence motifs that are not shared with other SF-2 helicases. However, the diversity of SF-2 sequences makes alignments based only on protein sequences unreliable, as they contain internal segments of varying length between the conserved motifs. Fortunately, there are now many crystal structures of RNA and DNA helicases that can be aligned structurally to identify the precise correspondence of sequence elements in diverse proteins. The alignment depicted in Fig. 2 was produced by incorporating structural alignments into conventional sequence-based alignment of SF-2 family helicases. This clearly reveals sequence features that are present only in RecQ helicases, both as variants of the canonical helicase motifs and as additional conserved motifs. One motif, the aromatic loop (following motif II), includes the highly conserved sequence CxSQWGHDFR; this was originally identified by Keck and co-workers as essential for DNA-coupled ATP hydrolysis, and may present a unique aspect of the catalytic mechanism of RecQ helicases [40]. The motif denoted IVa, AYHAG, is present in HEL308 and DEAD-box but not in DEAH-box helicases; this region has been implicated in DNA binding in HEL308 [41]. In addition, there are several RecQ-specific amino acids throughout the sequence whose significance remains to be determined.

The major distinguishing feature of RecQ proteins is the region termed RecQ-C-terminal (RQC), which is C-terminal to the core helicase domain. This region includes a Zinc-binding domain and a Winged-Helix domain, which can be identified in the sequences of

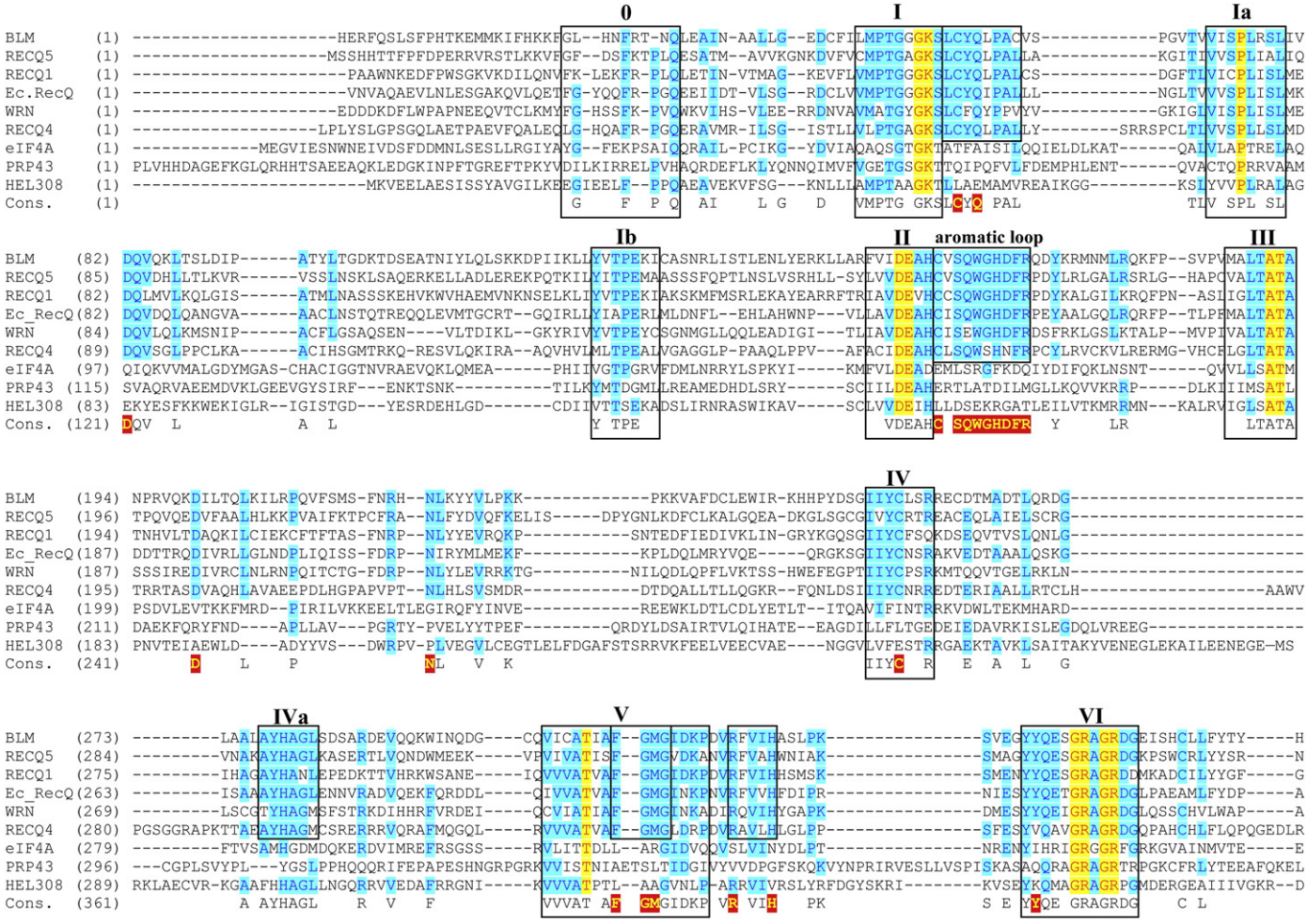

Fig. 2. Structure-based alignment of Superfamily 2 helicases: the RecA-like domains. The core ATPase domain of RECQ1 was used to search structural neighbours in the protein structure database (PDB) using the DALI server [93]; the alignments with superfamily 2 (SF-2) helicases were used to manually adjust a sequence-based alignment (VectorNIT/ClustalW) containing, in addition to the sequences from the DALI output, all human SF-2 helicases. The register of DEAH helicases had to be manually adjusted based on a structural overlay of RECQ1 (PDB ID:2v1x) and yeast PRP43 (PDB ID:3kx2). The alignment presented in the figure includes the five human RecQ proteins and *E. coli* RecQ, in addition to one representative each of the DEAD-box family (eIF4A), the DEAH-box family (s.c.PRP43), and the HEL308 protein. Residues highlighted in yellow are universally conserved, those highlighted in blue are common. Boxes extending through all sequences represent motifs conserved in all SF-2 families. Boxes encompassing only the RecQ-family sequences denote motifs that are highly conserved in this family but not in other SF-2 helicases. Residues that are uniquely conserved in RecQ helicases are highlighted in red at the consensus sequence line (note that the comparison used to define unique occurrence includes many sequences not shown in the figure).

most RecQ helicases with the notable exception of RECQ4. In addition to this conserved RCQ domain, some RecQ helicases have an auxiliary domain involved in substrate recognition, named the helicase-and-RNaseD-like-C-terminal (HRDC) domain. Together, these "extra" RQC and HRDC domains are important to confer distinct substrate specificities and enzymatic activities, in addition to the canonical helicase unwinding function. In the following paragraphs, we will focus on the description of the structural and functional information available for the core helicase domain and the two auxiliary domains of RecQ helicases. These three domains together contain the molecular code for the unique substrate specificity and multiple activities of RecQ proteins.

3. Helicase domain

The X-ray structures of the catalytic domains of only two RecQ helicases have been solved to date: the bacterial RecQ (from *E. coli*) and the human RECQ1 [42,43] (Fig. 3). The *E. coli* RecQ helicase catalytic core has been crystallized in two forms: a nucleotide unbound form at 1.8 Å resolution and a form bound to the slowly hydrolysable analogue of ATP, ATPγS, and Mn^{2+} at 2.5 Å resolution [42]. The structure of the human RECQ1 helicase bound to ADP and Mg^{2+} was solved at a resolution of 2.05 Å in the absence of nucleic acids [43]. At a first glance, the overall fold of the helicase domains of these two RecQ helicases is comparable to that of other helicases of the SF-2 family. The two RecA like domains contain the seven motifs characteristic of the helicase family, as mentioned above, and retain the general helicase fold. The superimposition of the nucleotide bound and unbound bacterial RecQ structures shows a slight relative rotation of the helicase domain in the nucleotide bound *versus* unbound state [42]. This rotation brings motif I to an open conformation, allowing the nucleotide to enter its designated cavity.

A structural alignment between the structures of RECQ1-ADP and *E. coli* RecQ-ATPγS bound forms shows a shift of more than 7 Å in the first of the two human RecA-like domains of RECQ1 compared to the same domain of the bacterial RecQ (Fig. 4). In line with this observation, multiple crystal forms of RECQ1 revealed that the relative orientation of the two RecA-like domains changes significantly supporting the notion that there is a high degree of flexibility between these two domains [43]. This relative motion could represent the conformational changes that occur during ATP-driven movement along the DNA; the magnitude of the shift is compatible with a 1–2 nucleotide step. In fact, a similar flexibility between the two RecA-like domains has been documented in various helicase structures and is supposed to be a key element involved in the ATP-dependent translocation activity of this class of enzymes [44,45].

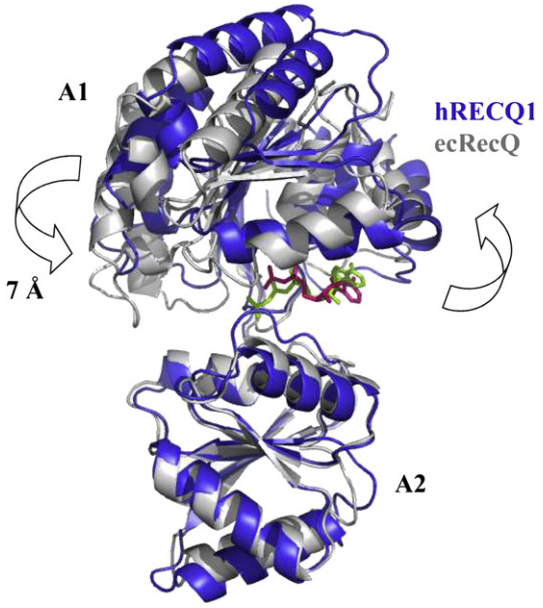

Fig. 4. ATPase interdomain flexibility. *E. coli* RecQ (grey) and human RECQ1 (blue) structures were superimposed using the A2 domains as a reference. The relative motions of A1 domains in the two homolog structures are indicated by the arrows. Bound nucleotides are shown as sticks.

The best examples are probably the *E. coli* SF-1 helicases PcrA and UvrD for which the different nucleotide bound states have been structurally studied in detail [44,45]. In these proteins, the binding of ATP leads to a reciprocal rotation of the two RecA-like domains and a closure of the cleft between domains 1A and 2A, allowing the enzyme to translocate on the 3′ single stranded product with a 3′-5′ polarity, like an inchworm. These observations were made possible by crystallizing the enzymes at different steps during the ATP-hydrolysis reaction coupled to partial unwinding of a synthetic duplex DNA. Concerning helicases that translocate with a 5′-3′ polarity, a recent structure of the *Deinococcus radiodurans* SF-1 helicase RecD2 in

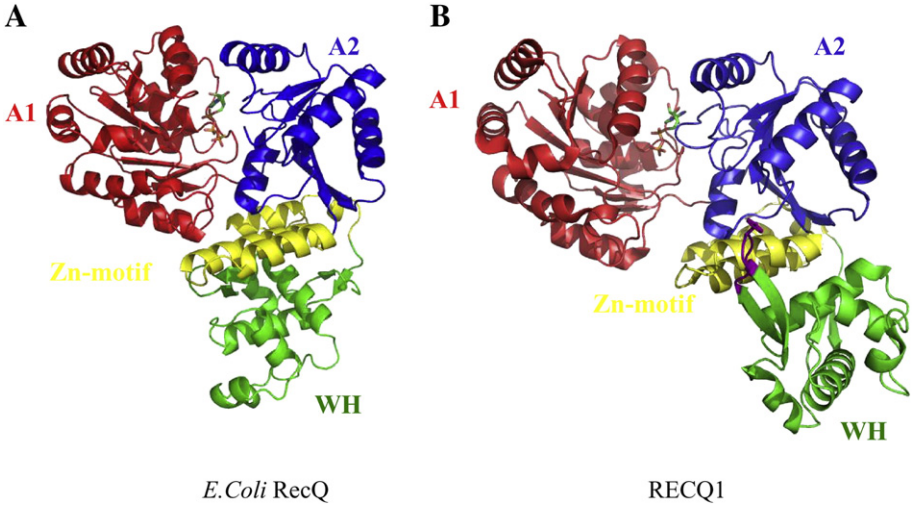

Fig. 3. Crystal structures of E. coli RecQ (A) and human RECQ1(B) helicase. The bacterial helicase is in complex with ATPγS (PDB ID: 1oyy) while the human enzyme with ADP (PDB ID: 2v1x) (both nucleotides are rendered as ball and stick in the pictures). The two proteins are highly similar and are color coded according their subdomains: core helicase A1 in red (residues 1–208 of RecQ and 63–281 of RECQ1), core helicase A2 in blue (residues 209–340 of RecQ and 282–418 of RECQ1), Zn-binding motif and helical hairpin in yellow (residues 341–406 of RecQ and 419–480 of RECQ1), WH domain in green (residues 407–516 of RecQ and 481–592 of RECQ1). The β-hairpin and Tyr564 at the tip of the hairpin are depicted in purple.

complex with ssDNA, in the presence and absence of ADPNP, indicated that this helicase translocates on the ssDNA in the 5′ to 3′ direction with a mechanism similar to PcrA, involving the closure of the 1A and 2A domains upon ATP binding [46]. However, the different translocation directionality is determined by the domains that tightly grasps ssDNA upon ATP binding. For 3′-5′ helicases such as PcrA, the domain 2A is tightly bound to DNA upon ATP binding causing the DNA to slide across the 1A domain to close the cleft. The balance is then reversed upon ATP hydrolysis. Conversely, for 5′-3′ helicases such as RecD2, the grip is tightest on the domain 1A upon ATP binding allowing the DNA to slide along the opposite domain. For a detailed description of the different translocation mechanisms we refer the readers to recent reviews [5,47,48].

Unfortunately a similar snap shot structural analysis using the different intermediates of the ATP-hydrolysis reaction is not available for any helicase of the SF-2 family as yet, leaving some degree of uncertainty on whether the SF-2 helicases adopt the same ATP-dependent translocation mechanism. However, high-resolution structural studies of SF-2 RNA helicases in different ligand bound states suggest a model for translocation where the two RecA domains can be visualized as a set of pincers with a highly conserved threonine residue that contact the phosphate backbone of the tracking strand [47,48]. The distance between the two pincers changes upon ATP binding and hydrolysis allowing the enzyme to translocate on the DNA lattice in the 3′-5′ direction. Further studies will be however required to understand if similar mechanism might also apply to other helicases of the SF-2 family, including the RecQ proteins.

The structure of the nucleotide bound form of *E. coli* RecQ shows that the adenine moiety of ATPγS is sandwiched between Tyr23 and Arg27 and hydrogen-bonded to Gln30 [42]. These three residues are part of an additional sequence element characteristic of RecQ helicases, called motif "0" [49], which is located at the N-terminus of motif I (Fig. 2). This motif is a RecQ-specific variant of the Q motif of DEAD-box helicases [50], and is well conserved in RecQ helicases from different organisms. Motif 0 of RECQ1 is composed of Leu89 that replaces the aromatic residue of the *E. coli* protein (Tyr23), Arg93 which is conserved among other RecQ helicases including the bacterial RecQ protein (Arg27 in *E. coli* RecQ), and by Gln96 which corresponds to Gln30 in the bacterial enzyme [43]. Gln96 is hydrogen bonded to the adenine moiety of ADP as observed for the *E. coli* RecQ. Mutagenesis studies confirmed that this motif is important for core helicase domain function. For example, mutation of the C-terminal Gln34 to Ala in RECQ5β significantly reduces ATPase activity, and the substitution of the same residue with Arg inactivates the ATPase and helicase function of murine BLM [25,51]. The same mutation is also listed among the naturally occurring mutations of *BLM* associated with Bloom's syndrome [11]. Moreover, budding yeast cells carrying this mutation in the *SGS1* gene show the same DNA-damaging agent sensitivity as that of an sgs1 deletion strain [52].

4. The RQC domain

The RecQ-C-terminal (RQC) domain is present in all members of RecQ subfamily, but human RECQ4 and its orthologs. It is composed of a zinc binding module and a helix-turn-helix fold, the so-called winged-helix (WH) domain. The three-dimensional structure of the whole RQC domain is described in the crystal structures of bacterial RecQ and human RECQ1 [42,43]. Moreover, the crystal structure of the isolated RQC domain of human WRN in complex with a DNA duplex has been solved while this review was in preparation, and the structure of the sole WH domain of WRN has been previously obtained in solution by NMR [53,54]. The structures of the zinc binding modules are practically identical between bacterial and human enzymes, where a single Zn^{2+} ion is coordinated by four Cys residues positioned on two anti parallel α-helices. The notion that this zinc module plays an important role in RecQ helicase function is supported by the fact that missense mutations affecting the Cys residues of the Zn^{2+}-binding pocket of BLM are found in Bloom's syndrome cases, and a similar mutation in the budding yeast *SGS1* gene confers enhanced DNA-damage sensitivity and a hyperrecombination phenotype [11,52]. The observation that a single amino acid substitution in the Zn^{2+}-binding domain of the human BLM and bacterial RecQ proteins generate variants that are either insoluble or very prone to degradation indicates that this motif is mainly required to confer protein stability [55–57]. In addition, the Zn^{2+}-binding module might be implicated in mediating DNA and/or protein interactions, as already indicated for other proteins that contain similar domains [58]; however, further studies will be required to evaluate this possibility.

The WH domain was previously demonstrated to act as a DNA-binding motif in many other proteins such as the transcription factors CAP and hRFX1, and the human DNA repair protein AGT [59–62]. Surprisingly, despite their low sequence identity, the superimposition of *E. coli* RecQ, RECQ1 and WRN WH-domains reveals a remarkably conserved fold (Fig. 5). However, the relative position of the WH-domains in the structures of RECQ1 and *E. coli* RecQ is different: in the first one it lies beneath the zinc biding module, while in the bacterial enzyme it lies perpendicular to the two RecA-like domains [43] (Fig. 3). A structural alignment of the structures of *E. coli* RecQ with the *E. coli* catabolite gene activator protein CAP co-crystallized with ds-DNA has led to the suggestion that the DNA duplex is located in a cleft between the WH- and the Zn^{2+}-binding domains [42]. However, in the structure of RECQ1 the different orientation of the WH-domain closes the cleft in which ds-DNA is supposed to bind in the bacterial counterpart. This might suggest a different DNA binding mode between the human and bacterial RecQ helicases (see below). On the other hand, we cannot rule out the possibility that the different orientations of the WH helix domains relative to the rest of the molecule could be due to the crystal packing process. Alternatively, they could also reflect different states accessible to both enzymes or diverse states derived from different mechanisms of action.

A remarkable feature that characterizes the WH of these RecQ helicases is an extended β-hairpin motif that carries a Tyr residue at the tip in the human RECQ1 protein [43] (Fig. 5). This β-hairpin is considerably shorter in *E. coli* RecQ and WRN where the Tyr residue at

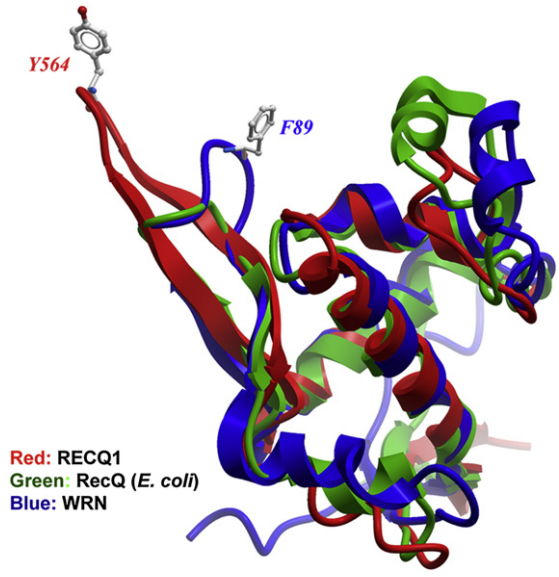

Fig. 5. Superimposition of WH domains. The WH domains of the RecQ-Ct regions of human RECQ1 (red; PDB ID: 2V1X), *E. coli* RecQ (green; PDB ID: 1OYW) and WRN (blue; PDB ID: 2AXL) are shown. The beta-hairpins are seen in the upper-left corner. Aromatic residues at the tip of the hairpin (Y564 in RECQ1 and F89 in WRN) are shown; there is no aromatic residue at the tip of the shorter hairpin of the *E. coli* enzyme.

the tip is substituted by His and Phe, respectively. Our mutagenesis studies show that the aromatic residue at the tip of the β-hairpin in human RECQ1 is required for DNA unwinding, as the simple substitution of Tyr to Ala is sufficient to abrogate the helicase activity of the protein almost completely [43]. Consistently, an analogous β-hairpin element was found to be essential for the unwinding activity of other helicase members of both the SF-1 and SF-2 family. The crystal structures of two SF-2 proteins, the archeal Hel308 from *Archaeoglobus fulgidus* and helicase NS3 of the hepatitis C virus, both show a β-hairpin structure between motifs V and VI of the helicase domain [41,63]. Moreover, the structure of Hel308 was solved in complex with a partially double-stranded DNA of 15 bp with a 3′ single-stranded tail of 10 nt and shows that the β-hairpin module is positioned exactly at the opening of the unwound tailed duplex [41]. The DNA bound structures of the SF-1 proteins, UvrD and PcrA, which have a tertiary structure formed by two RecA-like domains and two insertion domains, also show that the bound DNA duplex unwinds in front of a 'pin' located in domain 2A [44,45]. The current model suggests that the aromatic residue at the tip of the hairpin may act as a pin that abuts the end of the DNA duplex and hence promotes strand separation.

The fact that this hairpin is significantly shorter in *E. coli* RecQ and WRN suggests that these helicases might utilize a different mechanism to unwind DNA from that of RECQ1. Indeed we have shown that the His to Ala mutation at the tip of the hairpin in the bacterial RecQ, produces an enzyme able to unwind DNA with efficiency similar to the wild-type protein [43]. However, the recent structure of the RQC domain of WRN bound to a DNA duplex suggests that the β-hairpin plays an important role in the DNA strand separation activity of WRN [54]. Thus, we can speculate that RecQ helicases adopt a different mechanism for substrate recognition and unwinding, and that the β-hairpin motif represents a key structural element in the regulation of the enzymatic activity of this class of helicases.

A recently solved structure of a complex of the human RECQ1 with DNA supports this model (PDB ID: 2WWY; Pike et al, manuscript in preparation). Fig. 6 shows the crystal structure of RECQ1 lacking 48 and 35 residues at the N- and C-termini, respectively. Here, only one of the two RECQ1 subunits is shown even though this protein occurs as a dimer both in solution and in the crystal. Three important features emerge from this structure. First, the 3′ single-stranded DNA tail extends across the two RecA domains, as seen with SF-1 helicases and with the HEL308. Second, the duplex DNA is bound to the winged-helix (WH) domain, through interactions with the sugar-phosphate backbone. Third, the β-hairpin motif is positioned at the point of strand separation. This is consistent with the prediction that the hairpin acts directly to couple the ATP-driven tracking along the ssDNA tail to DNA strand separation.

5. The HRDC domain

The helicase-and-RNaseD-like-C-terminal (HRDC) domain plays an important role in DNA substrate recognition. This domain is present in several-but not all-members of RecQ subfamily. For example, among the human RecQ helicases, only BLM and WRN possess the HRDC domain. Interestingly, some organisms, such as *Rhodobacter sphaeroides*, possess two HRDC repeats and others, such as *Deinococcus radiodurans*, *Neisseria meningitidis*, and *Neisseria gonorrhea*, contain three repetitions of this domain. Structural and biochemical studies indicate that this scaffold is the most variable in the RecQ family and it is involved in substrate binding as well as in mediating protein–protein interactions [64].

The structures of the isolated HRDC domains from *E. coli* RecQ, *Deinococcus radiodurans* DrRecQ, and human WRN have been solved by X-ray crystallography [65–67] (Fig. 7). In addition, NMR studies have also provided structural data on the HRDC domain from *S. cerevisiae* Sgs1 [68]. The HRDC domain of Sgs1 was the first to be structurally studied and turned out to be similar in conformation-although not in its primary sequence-to domain 1B of bacterial DExx-box helicases, such as *E. coli* Rep and PcrA helicases (both members of the SF-1 family), and to the N-terminal domain of human DNA polymerase β [68]. The HRDC structure of Sgs1 is composed of five α-helices and one 3_{10}-helix packed together, and it is thought to have a role as an auxiliary domain involved in substrate recognition, similar to the 1B module of SF-1 helicases. In this regard, a patch of basic residues of HRDC Sgs1 was suggested to be involved directly-or indirectly-in substrate binding via electrostatic interactions in a similar fashion to the DNA binding site of DNA polymerase β (Fig. 7). This basic patch is also present in the primary sequence of the HRDC module of *E. coli* RecQ, but not in the sequences of HRDC's of BLM and WRN suggesting that other electrostatic interactions may be involved in the recognition of different substrates or protein cofactors. Consistent with this observation, in vitro assays demonstrated that the purified HRDC domain of Sgs1 has a different substrate specificity from the HRDC domain of other RecQ helicases in that it is able to bind, even if with a weak binding constant, both ssDNA and partially double-stranded DNA duplexes with 3′ single stranded overhangs of 6 and 8 nt [68].

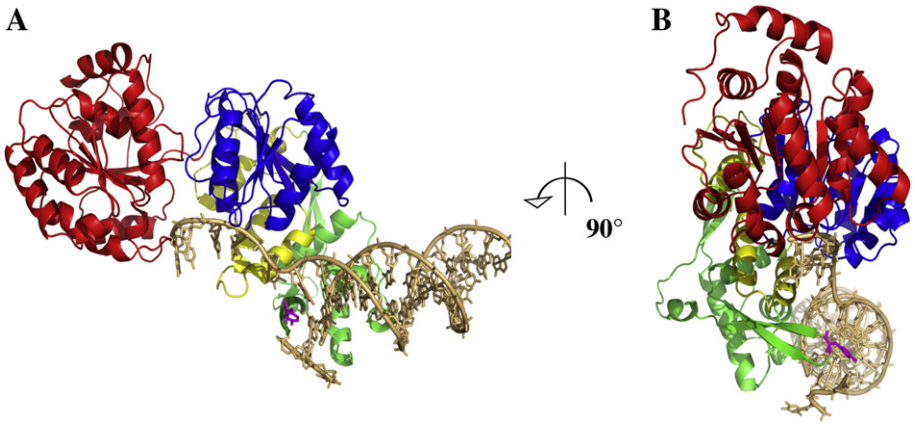

Fig. 6. Crystal structure of a complex of human RECQ1 with a DNA substrate (PDB ID: 2wwy). A crystal structure of human RECQ1 (aa 48–616) bound to tailed-duplex DNA was solved at 2.9 Å resolution (Pike et al., manuscript in preparation). The complex is represented in the canonical front view (A) with the dsDNA interacting with the WH domain and the ssDNA positioned between the two RecA-like domains, and rotated of 90° to the right (B) to show the critical position of Tyr564 located at the opening of the double stranded substrate. The RECQ1 molecule is colored to indicate the domain structure: RecA-like domains 1 (blue) and 2 (red); the Zn2+-binding domain (yellow); and the WH domain (green); the β-hairpin motif of the WH domain is shown in purple.

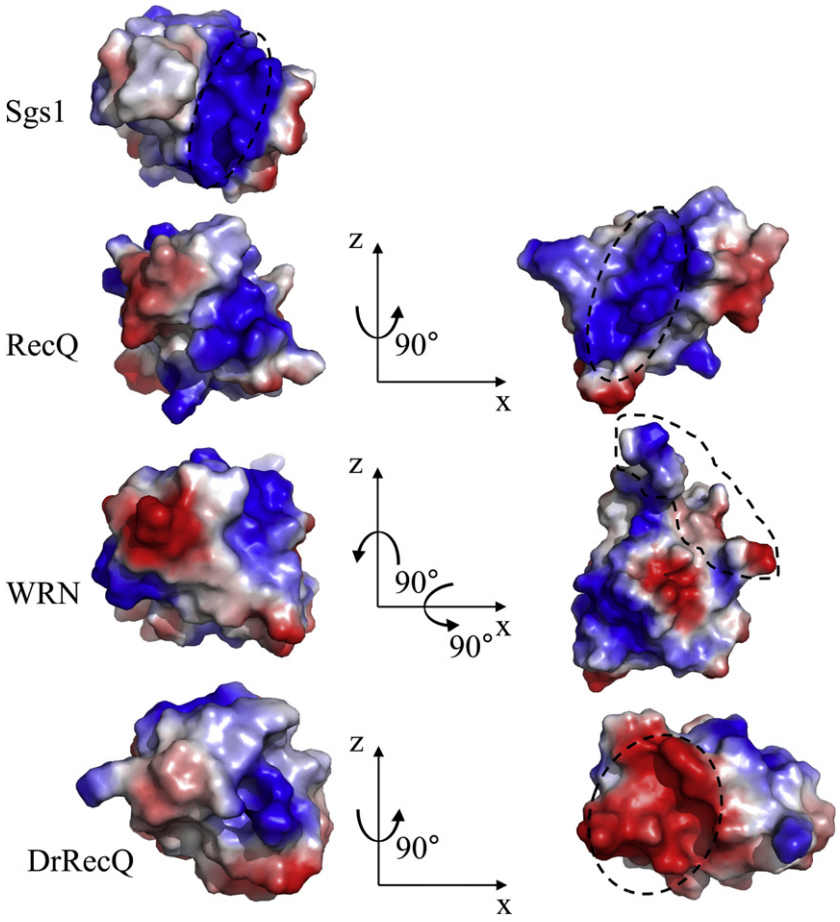

Fig. 7. Surface variability between HRDC domains of RecQ helicases. The electrostatic surface potentials of the HRDC domains of Sgs1, *E. coli* RecQ, WRN, and DrRecQ are shown. The left column shows the four HRDC domains in the same orientation. In the right column, the HRDC domains of *E. coli* RecQ, WRN, and DrRecQ are rotated relative to the HRDC domain of Sgs1 to show the most important features of each domain. Highlighted with dashed circles are: the positive patch of residues involved in DNA binding in the HRDC domains of Sgs1 and *E. coli* RecQ; the additional N- and C-terminal extensions that characterize the HRDC domain of WRN; the acidic path of residues possibly involved in substrate recognition in the HRDC domain of DrRecQ. Electrostatic surface potentials have been calculated using the Pymol software (Delano W.L. (2002) The Pymol Molecular Graphics System. DeLano Scientifics, San Carolos, CA).

The 2.2 Å structure of the HRDC domain from *E. coli* has a scaffold similar to HRDC from Sgs1 [65]. However, it preferentially binds ss-DNA in a mode similar to the 1B domain of SF-1 proteins, rather than the binding mode observed in Sgs1 and DNAP β [49,65]. The ss-DNA binding site is composed of one aromatic residue, Tyr555, and several positively charged residues that form the basic patch involved in the recognition of the negatively charged DNA backbone (Fig. 7). Interestingly, a single missense mutation-Tyr555 to Ala-in the full length bacterial RecQ drastically reduces the binding affinity towards a partially double-stranded DNA with a 3′ single-stranded overhang of 12 nt and enhances the affinity for Holliday junctions DNA [65].

The HRDC from WRN was recently crystallized in different crystal forms [67]. The combination of biochemical and structural studies revealed important and unexpected peculiar characteristics. The domain is composed of the conventional five α-helices and one 3_{10}-helix observed in its Sgs1 homolog, but it also possesses additional N- and C-terminal extensions that are missing in the HRDC structures of other RecQ helicases. The surface electrostatic potential does not include the basic residues observed in HRDC from Sgs1 and *E. coli* RecQ which should interact with the acidic DNA backbone (Fig. 7). Hakoshima and co-workers showed that the HRDC domain of WRN does not bind any DNA forms using *in vitro* binding assays [67]. Thus, the authors suggest a role of this domain in mediating protein–protein interactions with some of the known WRN interactors, such as the tumor suppressor protein p53, the homologous recombination mediator RAD52, and the p50 subunit of DNA polymerase δ. However, another study reports that a fragment of WRN including the HRDC and some additional residues at C-terminus (fragment 1072–1432) binds Holliday junctions and forked DNA structures but not ssDNA [69].

Even if the structure of the HRDC from BLM is absent, we know that this domain is critical for the efficient unwinding of Holliday junction substrates and that the HRDC from WRN cannot complement this function consistent with the fact that the two domains share only 19% homology. Furthermore, the lysine-1270 residue of BLM, which resides in the HRDC domain, plays an important role in the double Holliday junction dissolution activity of BLM, an activity that is highly specific for BLM among human RecQ helicases [70,71].

The bacterial DrRecQ from *D. radiodurans* contains three HRDC domains. The domain HRDC3 has recently been crystallized at a resolution of 1.1 Å [66]. Again, even though there is low sequence similarity between the HRDC3 domain of *D. radiodurans* and that of other RecQ helicases, the overall scaffold is similar to that of other HRDCs. However, HRDC3 presents novel and peculiar features that distinguish this HRDC domain from that of other helicases. These features refer mainly to the surface potential of the domain and to the ability to bind different DNA substrates (Fig. 7). For example, an acidic patch, rather than the basic patch observed for Sgs1 and *E. coli* RecQ, seems to be important for binding and unwinding certain DNA structures. Moreover, some basic residues homologus to the ones involved in DNA binding in *E. coli* RecQ, Sgs1 and BLM are also present in DrRecQ HRDC, but they seem to play a minor role in DNA binding, as demonstrated by their substitutions [66].

Collectively, these studies indicate that the HRDC domain plays an important role both in conferring some specific enzymatic activities to the individual RecQ enzymes and in DNA structure-specific recognition. In addition, it may mediate some protein–protein interactions. The question of how the HRDC domain interacts functionally with the other two conserved regions of RecQ enzymes (the core helicase and the RQC domain) and, as a result, modulates the specific enzymatic properties of the individual helicases, is still open.

6. The exonuclease domain

The N-terminal region of human WRN is characterized by a clearly-defined exonuclease domain of approximately 200 aa that promotes nucleotide digestion with a 3′ to 5′ polarity [29,30,72]. Human WRN and its orthologs in other organisms, such as *X. laevis* FFA-1, are the sole RecQ family proteins possessing this additional exonuclease activity. Several crystal structures of the WRN exonuclease domain (region 38–236) have been determined at up 2.0 Å resolution [73]. This domain is characterized by an αβ fold consisting of a central β-sheet surrounded by seven α-helices (Fig. 8). The WRN-exo structure highly resembles the structures of DnaQ exonucleases from bacteria and archea, which include the 3′-5′ proofreading domain of *E. coli* DNA polymerase I, known as the Klenow Fragment (KF-exo). In line with this observation, the metal-ion complex structures of WRN-exo indicate that the catalytic mechanism of phosphodiester bond hydrolysis involves two metal ions as already suggested for other KF-exo. However, although the active sites residues are strictly conserved between WRN-exo and KF-exo, major differences reside in their respective substrate binding sites.

The N-terminal exonuclease domain can function independently of the rest of WRN polypeptide and displays a 3′ to 5′ exonuclease activity similar to the full-length protein [29]. Interestingly, the full-length WRN protein was shown to form multimeric structures ranging from dimers to hexamers in solution [34,36]. This ability to form higher-order oligomeric structures is retained by the exonuclease domain of WRN in that a recombinant fragment encompassing residues 1–333 elutes at a size consistent with a trimeric structure from a Superdex S-200 size-exclusion chromatography column [74]. Moreover, atomic force microscopy analysis of a 171-amino acid fragment of WRN comprising the exonuclease domain of the enzyme revealed a trimer–hexamer equilibrium in the absence of DNA, with the equilibrium being significantly shifted toward the hexamer form upon interaction with DNA or with the polymerase clamp loader PCNA [75]. Thus, we can speculate that WRN hexameric ring formation is required for optimal exonuclease activity. Consistent with this idea, Perry et al. propose a hexameric ring model for the WRN-exonuclease domain where a central cavity of appropriate size to accommodate a dsDNA probe contains the active site of the enzyme [73]. These hexamers might be also involved in the interaction with the Ku70/80 heterodimer previously shown to stimulate the exonuclease activity of WRN [76,77]. The transient stacking of Ku with the WRN hexamers at dsDNA ends would prevent the falling of WRN from broken DNA ends and allow an efficient regulation of the WRN-end processing activity during DNA non-homologous end-joining reactions.

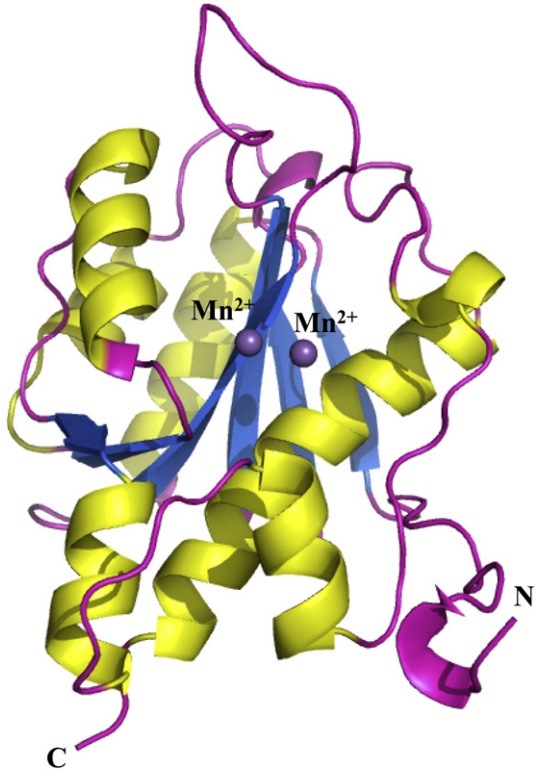

Fig. 8. WRN exonuclease domain. The structure of the WRN exonuclease domain (residues 38–236) was solved at 2.4 Å resolution (PDB ID: 2FBV). The α helices are colored in yellow and β sheets in light blue. The two spheres represent two Mn^{2+} ions.

7. RecQ helicase oligomers and strand annealing

Recent studies demonstrated that several RecQ enzymes are able to promote the annealing of complementary single stranded DNA molecules [24–28]. This activity might be required to promote either branch migration of DNA junctions or the regression of stalled replication forks, which are characteristic activities of several RecQ helicases and which might be facilitated by 'active' DNA strand annealing. The exact function of the annealing activity of RecQ helicases *in vivo* has, however, yet to be demonstrated.

Using a combination of size exclusion chromatography and transmission electron microscopy approaches, we demonstrated that different oligomeric forms of the human RECQ1 helicase are associated with the two opposite enzymatic activities: a higher-order oligomeric form is associated with strand annealing, while a smaller form consistent with monomers or dimers is responsible for DNA unwinding [37]. The equilibrium between these two forms is controlled by ATP binding in that the addition of the nucleotide induces the dissociation of the higher-order oligomeric structures in the presence and absence of DNA. Analytical ultracentrifugation experiments indicated that the higher-order oligomeric form is probably a tetramer rather than a pentamer or an hexamers as was originally inferred from size exclusion chromatography experiments (Lucic et al., manuscript in preparation). In line with this observation, preliminary fitting experiments using the coordinates of the crystal structure and the density maps of RECQ1 derived by electron microscopy show that the higher-order oligomers might represent tetramers. The notion that RecQ helicase multimers are required for strand annealing is also supported by the observation that BLM mutants lacking strand annealing activity fail to form higher-order protein–DNA complexes in gel retardation assays, suggesting that oligomerization is required for the strand annealing function of BLM [24]. Electron microscopy experiments have shown that the full-length BLM protein can form hexameric ring-like structures in the absence of ATP and DNA [35]. However, the same study also described the presence of four-fold symmetric structures that might represent an oligomeric form distinct from the hexameric ring (such as a tetramer or an octamer). In the case of WRN, a C-terminal fragment located between the RQC and the HRDC domains of the protein retains single strand annealing activity, which correlates with the formation of hexamers and trimers during size-exclusion chromatography experiments [36].

The mechanism that RecQ helicases use to promote the pairing of complementary single stranded DNA fragments is still unknown,

mainly because high resolution structural data on the higher oligomers are missing. The electron microscopy maps of the higher-order RECQ1 oligomers show the body of the helicase to be arranged in two ring-like densities interconnected by diagonally extending densities to a larger middle ring-like structure [37]. The three rings seem to form a hollow channel with an inner diameter of approximately 20 Å. Although highly speculative, this central pore could accommodate dsDNA with the diagonally slatted densities aiding ssDNA to wrap around the oligomer and into the central channel where it could associate with the other strand that threads through the full length of the channel.

A different model might be derived from the structure of the recombination protein RAD52, which is among the best characterized single strand annealing proteins. This structure shows an undecameric subunit ring with a positively charged groove running along the surface of the ring [78,79]. The current model of ssDNA annealing suggests that multiple RAD52-DNA complexes with the ssDNA bound to the exposed groove of the ring associate to facilitate the pairing of the bases and the single strand annealing reaction. Even though there are no obvious structural similarities between the single strand annealing domain of RAD52 and the structures of the catalytic domains of human RECQ1, one might assume that this is because the domain required for ssDNA annealing is missing in the structure of the truncated RECQ1$^{49–616}$ helicase. However, we think this possibility is highly unlikely considering that the low-resolution structure of higher-order oligomeric form of RECQ1 obtained by electron microscopy does not show any obvious similarity with the structure of the RAD52 complex.

The bacterial RecO and bacteriophage T4 UvsY represent another example of proteins that mediate the annealing of complementary ssDNA complexed with their cognate single-strand DNA binding proteins, SSB and gp32 [80]. Also in this case, there is no obvious sequence or structural similarity between these two proteins and RAD52, even though they share a similar function [81,82]. Interestingly, the interaction of RecO with the RecR protein reduces the annealing activity of RecO and promotes the displacement of the SSB protein from ssDNA by RecA suggesting that protein:protein interactions regulate the balance between the distinct activities of the RecO protein, possibly by inducing conformational changes in the RecO molecule that either expose or hide regions important for annealing [83,84].

Our mutagenesis studies indicated that the N-terminal region (residue 1–56) of RECQ1 is necessary for higher-assembly state formation and confirmed that the truncated protein is unable to promote the ssDNA annealing reaction, supporting the notion that higher oligomers are required for strand annealing [19]. We have previously mentioned the fact that the RECQ1 structure is characterized by a prominent beta-hairpin located in the WH domain of the protein and that the deletion of residues flanking the Tyr at the tip of the hairpin-or the simple Tyr to Ala substitution-abolishes DNA unwinding [43]. Unexpectedly, when we tested the impact of the same hairpin mutations on the DNA strand annealing activity of RECQ1$^{49–616}$ we saw that these mutants, even though they do not form higher oligomers in solution, retain DNA strand annealing activity comparable to that of the full-length RECQ1 (Lucic et al., manuscript in preparation). It is possible that the strand annealing activity of the hairpin-mutated RECQ1$^{49–616}$ is unrelated to the annealing activity of the full-length protein, and instead is a consequence of the defect in strand separation activity. However, an intriguing possibility is that the hairpin may play an important role in the regulation of the strand annealing activity of wild-type RECQ1. If this is the case, it is tempting to speculate that protein oligomerization may be required to reorient the Tyr residue and create a conformation able to promote the annealing reaction.

8. RecQ helicase oligomers and HJ resolution

Several RecQ helicase proteins, including human BLM, WRN, and RECQ1, and the yeast homolog Sgs1 were shown to selectively bind Holliday junction structures and to promote ATP-dependent branch migration [22,23,85]. Given that RecQ helicases mainly act during DNA replication, several lines of evidence suggest that this activity is required to avail the resolution of potentially recombinogenic molecules that arise at sites of stalled replication forks. RecQ helicases would act as anti-recombinases by catalyzing reverse branch migration of Holliday junctions that may form at stalled or disrupted replication forks, thus leading to restoration of an active replication fork.

The fact that BLM, WRN, and RECQ1 form oligomeric structures in solution stimulates the provoking thought that these RecQ helicases may promote branch migration with a mechanism similar to the oligomeric RuvAB branch migration motor. Two RuvA tetramers bind the junction and promote the loading of two RuvB hexamers on the two arms of the junction [86–92]. Indeed, mutagenesis studies demonstrated that a truncated form of the RECQ1 helicase lacking the first 48 residues at the N-terminus is unable to form higher oligomeric structures and to catalyze Holliday junction resolution [19]. Thus, the higher-assembly state formation promoted by the N-terminus, or the N-terminus itself, is essential for the ability of the protein to resolve Holliday junctions. The crystal structure of the RECQ1-DNA complex (Fig. 9) shows simultaneous binding of two ds:ss DNA junctions.

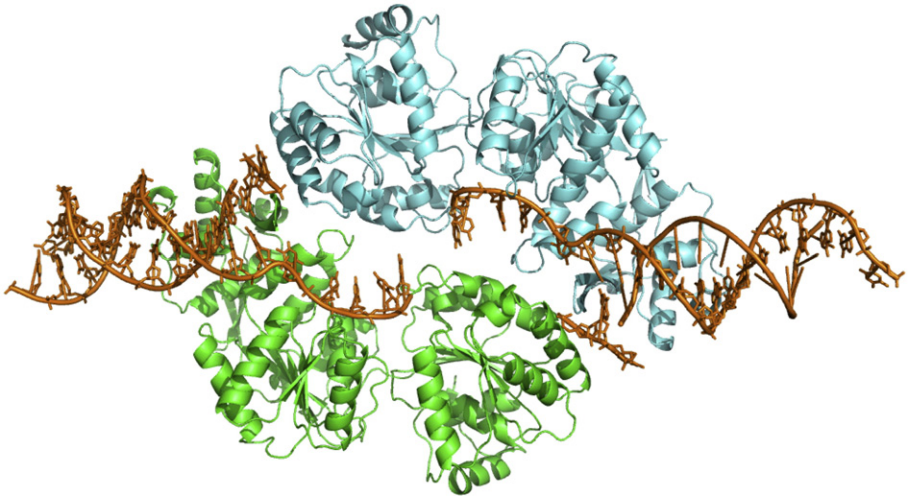

Fig. 9. Structure of the RECQ1 dimer in complex with DNA. The recently solved structure of RECQ1 in complex with a DNA substrate (PDB ID: 2wwy), contains two molecules in the asymmetric unit. The position of the two RECQ1 molecules bound to DNA might provide a hint of the structural mechanism by which this enzyme is able to resolve Holliday Junctions substrates.

Although this simultaneous binding may be a feature of crystal arrangement, it may hint at the way a four-way junction may be bound to a RECQ1 oligomer (the location of additional RECQ1 subunits in such a model remains to be determined).

The loading of RuvB hexamers on the two arms of a Holliday junction DNA requires the presence of the RuvA tetramer that specifically binds the junction. RecQ helicases are however able to specifically recognize the cross-over of the X-junction without the help of other accessory proteins even though other factors might be required to increase the specificity of this interaction or to promote branch migration in a directional fashion. Further studies will be required to understand if RecQ helicases are 'true' Holliday junction specific branch migration proteins such as RuvAB or if they act as structure specific helicases that have the ability to translocate branched structures by a different mechanism.

9. Concluding remarks

The multiple functions of the RecQ helicase genes are just beginning to emerge. How the five human RecQ homologs interplay to maintain the integrity of the genome is however still a mystery. In vitro and cellular studies suggest RecQ helicases play distinct functions in the stabilization and repair of damaged DNA replication forks, replication initiation, telomere maintenance, base excision repair, homologous recombination, and also DNA damage checkpoint signaling. These multiple functions are possible thanks to a combination of different enzymatic activities that differentially characterize each RecQ helicase. The ability to unwind various DNA structures in addition to "more conventional" B-form DNA duplexes also suggests that RecQ helicases possess unique structural features that distinguish them from any other canonical helicase pertaining to the SF-1 or SF-2 family. In addition, RecQ helicases are characterized by a unique regulatory mechanism that promotes the switch from a DNA unwinding mode to a strand annealing or branch migration mode. Is this regulatory mechanism controlled by protein oligomerization? Our studies suggest that this is the case for RECQ1 and recent reports suggest that the same conclusion holds for WRN and BLM. Further studies will be however required to understand if this is a general mechanism shared by all other RecQ helicases. In this regard, new structures of RecQ proteins in complex with their different DNA substrates or protein co-factors would be highly informative. These studies would not only be essential to understand if RecQ helicases share similar mechanisms to perform their multiple enzymatic activities, but also would provide an invaluable tool for the rational design of specific- or pan-RecQ helicase inhibitors of potential use in cancer therapy.

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