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Bioinformatic analysis of RecQ4 helicases reveals the presence of a RQC domain and a Zn knuckle

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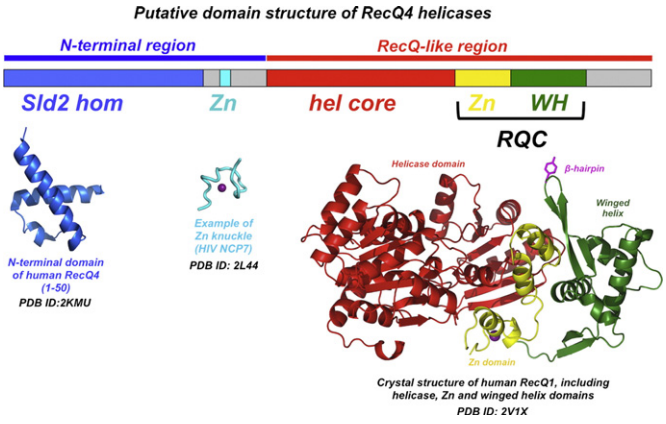
HIGHLIGHTS

- The similarity between yeast Sld2 and the N-terminal region of RecQ4 extends to the entire Sld2 sequence.
- The N-terminal domain of RecQ4 contains a Zn knuckle.
- A putative RQC domain can be identified in RecQ4, including a Zn domain and a winged helix domain.

GRAPHICAL ABSTRACT



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ABSTRACT

RecQ helicases play essential roles in the maintenance of genome stability and contain a highly conserved helicase region generally followed by a characteristic RecQ-C-terminal (RQC) domain, plus a number of variable associated domains. Notable exceptions are the RecQ4 helicases, where none of these additional regions have been described. Particularly striking was the fact that no RQC domain had been reported, considering that the RQC domain had been shown to play an essential role in the catalytic mechanism of most RecQ family members. Here we present the results of detailed bioinformatic analyses of RecQ4 proteins that identify, for the first time, the presence of a putative RQC domain, including some of the key residues involved in DNA binding and unwinding. We also describe the presence of a novel "Zn knuckle" domain, as well as an additional Sld2-homology region, providing new insights into the architecture, function and evolution of these enzymes.

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1. Introduction

RecQ proteins belong to an ubiquitous family of DNA helicases that are able to unwind unconventional DNA substrates and play an essential role in the maintenance of genome stability by acting at the interface between DNA replication, recombination and repair [1–4], although their exact cellular roles are still somehow unclear. They contain a highly conserved helicase domain generally followed by an associated C-terminal domain known as RQC domain [5] (Fig. 1A). Crystallographic studies on the *Escherichia coli* RecQ [6] and the human RecQ1 helicases [7] have shown that the RQC can be structurally divided into two independent regions: a Zn-binding and a winged-helix fold (Fig. 1B). A number of additional domains may also be associated with the "RecQ core"; the most common is the C-terminal HRDC domain, a helical bundle found in a number of helicases and RNases (Fig. 1A).

Whereas bacteria and yeasts possess a single RecQ helicase, most vertebrates comprise various paralogues, with five RecQ-like proteins present in human cells, known as RecQ1, Bloom, Werner, RecQ4 and RecQ5 (Fig. 1A). Mutations in the gene encoding three of the five human RecQ paralogues are linked to defined genetic disorders associated with genomic instability; namely, Bloom syndrome, Werner syndrome, and Rothmund-Thomson, RAPADILINO and Baller-Gerold syndromes [2]. The different clinical features of these disorders support the notion that these human helicases have related but distinct functions in cells.

The human RecQ4 helicase (also known as RecQL4) [8,9] comprises 1208 amino-acid residues, including a helicase core roughly located between residues 480 and 820 (Fig. 1A). Initial reports on the biochemistry of RecQ4 proteins suggested that the protein did not possess any DNA helicase activity, despite the conservation of the SF2 helicase motifs [10,11]. A possible explanation of this intriguing observation was the unusual absence of a RQC domain, which has been shown to be essential for the catalytic activity in other RecQ proteins [12,13,7]. More recently, various laboratories have demonstrated that the recombinant protein is an active DNA helicase. A report [14] suggests that the helicase activity is associated not only with the helicase core but also the N-terminal domain, despite the lack of any obvious ATPase or helicase motif. Others show a weak but reproducible helicase activity associated to the helicase core only [8,15,16].

A long N-terminal domain is found upstream of the helicase domain, sharing no homology with other RecQ helicases. Some similarity to the yeast DNA replication initiation factor Sld2 has been detected for the first 150 amino-acid residues [17] (Fig. 1A). Phosphorylation of the yeast Sld2 by the S-phase cyclin-dependent kinase constitute an essential step in the initiation of DNA replication in yeast [18–20]. The hypothesis of an essential role in the initiation of DNA replication for RecQ4 has been recently confirmed by a number of in vivo studies in *Xenopus* [21,17], *Drosophila* [22,23] and mammalian cells [24–26].

Although mutations in human RecQ4 give rise to three distinct genetic disorders (Rothmund-Thomson, RAPADILINO and Baller-Gerold syndromes, [27,28]), the protein is one of the less characterised members of the RecQ family; detailed knowledge about its structure, function and cellular role is still patchy and that makes it difficult to interpret the effect of the disease-causing mutations. Through detailed bioinformatic analyses we have identified a series of novel features in the sequence of RecQ4, including a Zn knuckle and a putative RQC domain, which shed light on the structure and architecture of the protein (Fig. 1C).

2. Materials and methods

Database searches were carried out using the BLAST [29] algorithm, using the human RecQ1 and RecQ4 sequence as search model and default parameters. Multiple sequence alignments were generated with the ClustalW [30] and MUSCLE programmes [31] and manually modified to account for the positioning of the secondary structure elements and the novel features emerging from the fold recognition analysis.

A variety of threading/fold recognition algorithms were also used to identify or confirm the presence of structural homology. These include the Protein Fold Recognition Server Phyre [32], the profile–profile alignment and fold-recognition server FFAS03 [33] as well as the homology detection and structure prediction server HHpred, based on the pairwise comparison of profile hidden Markov model [34].

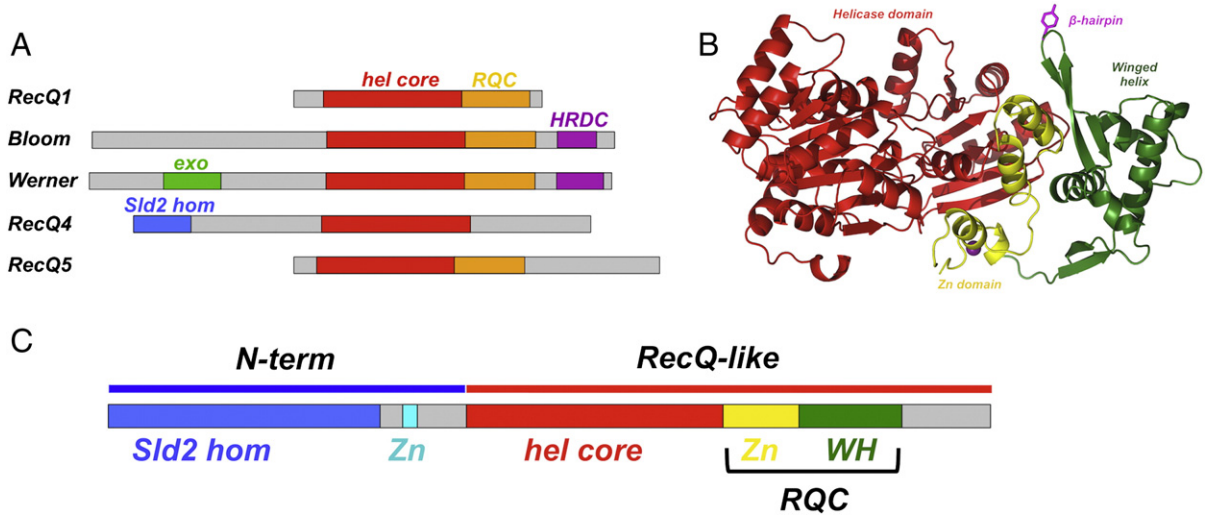

Fig. 1. The architecture of RecQ helicases. A) A schematic representation of the established architecture of the five human RecQ proteins. The helicase core domain (in red) is generally followed by a RQC domain (in orange), with the exception of RecQ4. In some members of the family are present additional domains, such as the C-terminal HRDC domain (purple) in Bloom and Werner helicases, an exonuclease domain in the N-terminal part of Werner (light green), an Sld2-homology region in RecQ4 (blue). B) The crystal structure of the core of RecQ1 [7] (PDB ID: 2V1X) showing the helicase domain (red), as well as the Zn-domain (yellow) and winged helix domain (green) which form the RQC. The Zn atom is shown in purple; the key tyrosine at the tip of the β-hairpin is shown as ball-and-stick in magenta. C) The proposed architecture of RecQ4 proteins, based on our bioinformatics analysis. The Sld2-homology domain (blue) extends much further than the first 150 residues; the location of a putative Zn knuckle is shown in cyan. A Zn-motif (yellow) and a winged helix fold (green) follow the helicase domain (red) forming a RQC domain.

3. Results

3.1. The Sld2-homology region encompasses the entire N-terminal region

A pattern of sequence similarity was originally reported between the N-termini of RecQ4 helicases and fungi Sld2 covering the first 150 amino-acid residues, of which the first 50 show the highest level of conservation [17], suggesting that RecQ4 represents the higher eukaryotes counterpart of Sld2. Although a NMR structure of the first 50 amino acids folds into a helical bundle reminiscent of a homeodomain-like fold [35] (PDB ID: 2KMU), sequence analysis indicates that the rest of the N-terminus contains large regions that are likely to be partially disordered.

We have detected an additional region that shows a sequence homology between the RecQ4 and the C-terminus of Sld2 proteins. Starting from *Shizosaccharomyces pombe* Sld2 as a search model the Position-Specific Interactive (PSI) BLAST [29], allowed the identification, on the second iteration run, of a weak but significant similarity with RecQ4 proteins extending to the end of Sld2. More specifically it identifies a similarity over the entire Sld2 sequence with RecQ4 from primates (*Callithix jaccus*, E-value = 7e−15), fishes (*Takifugu rubripes* E-value = 5e−14, *Danio rerio*, E-value = 2e−11), rodents (*Rattus norvegicus* and mouse, E-value = 3e−9), ruminants (*Bos taurus*, E-value = 6e−8), marsupials (*Sarcophilus harrisii*, E-value = 1e−7). A region towards the end (residues 343–368 in human RecQ4 and 424–453 in *Saccharomyces cerevisiae* Sld2; Fig. 2A) appears more conserved in the alignments. These two conserved regions are separated by roughly 300–500 less conserved residues; however, these intermediate regions are also poorly conserved within each family, and in most cases are predicted to be disordered or partially disordered. We therefore suggest that the similarity between Sld2 and RecQ4 encompass the entire Sld2 sequence, rather than simply the N-terminal region. Mutagenesis studied carried out on yeast Sld2 identified three lysine residues implicated in binding to the origin DNA [36]: of those, $K44^{Sld2}$ and $K50^{Sld2}$ are located in the N-terminal 50 residues and are conserved in RecQ4. The third residue, $K438^{Sld2}$, is located in this newly identified conserved region: within RecQ4 orthologues K438 is a conserved asparagine, but is surrounded by a number of highly conserved aromatic and positively charged residues, which could possibly be involved in binding origin DNA.

3.2. RecQ4 contains a Zn-knuckle upstream the helicase domain

We have identified a conserved Cys-rich motif [(S/T)**C**(F/Y) x**C**Gxx**H**WAxQ**C**] (residues 394 to 425 in the human sequence, Fig. 2B) which shows some homology with a number of Zn-binding domains. Running BLAST [29] for the region included between residues 380 and 450 of RecQ4 shows some homology to a number of retrotransposon proteins and gag-polyproteins of putative viral origins, as well as other proteins containing Zn knuckles. Fold recognition/structure prediction algorithms such as HHpred [34], Phyre [32] and FFAS03 [33] also detect strong similarities with Zn-binding folds variously classified as "retrovirus Zn-finger like" or "Zn knuckles". All retroviral nucleocapside proteins contain one or two copies of Zn knuckles; the best studied are the C-terminal Zn motifs of the HIV-1 nucleocapside protein NCp7 where Zn^{2+} binding has been shown to be necessary for most protein function [37]. The family also contains members involved in eukaryotic gene regulation: for example, the RNA binding protein Lin-28 contains two Zn knuckles that are involved in binding and processing members of the pre-let-7 family of miRNAs [38].

Of the RecQ4 paralogues analysed, the human sequence is unusual in having the second cysteine substituted by an asparagine (CNHC rather than CCHC, Fig. 2B). Interestingly, the bovine sequence has the histidine substituted by a glutamine (CCQC). Whether the human and bovine RecQ4 regions do bind Zn in the absence of the canonical cysteine residue is an open question.

3.3. RecQ4 contains a putative RQC domain

Whereas the large majority of RecQ helicases (including human RecQ1, Bloom, Werner and RecQ5) contain a RQC domain, which is an integral part of the catalytic core (Fig. 1A), no description of such domain is reported for RecQ4 proteins, nor is the domain identified by sequence/fold recognition servers. However, careful multiple sequence alignments between RecQ4 and other RecQ proteins, together with some indication from structure prediction/fold recognition algorithms such as HHpred [34] and FFAS03 [33], support the presence of a RQC domain in RecQ4 proteins (Fig. 3). The homology is more pronounced with human RecQ1, but some residues are conserved throughout most of the RecQ proteins.

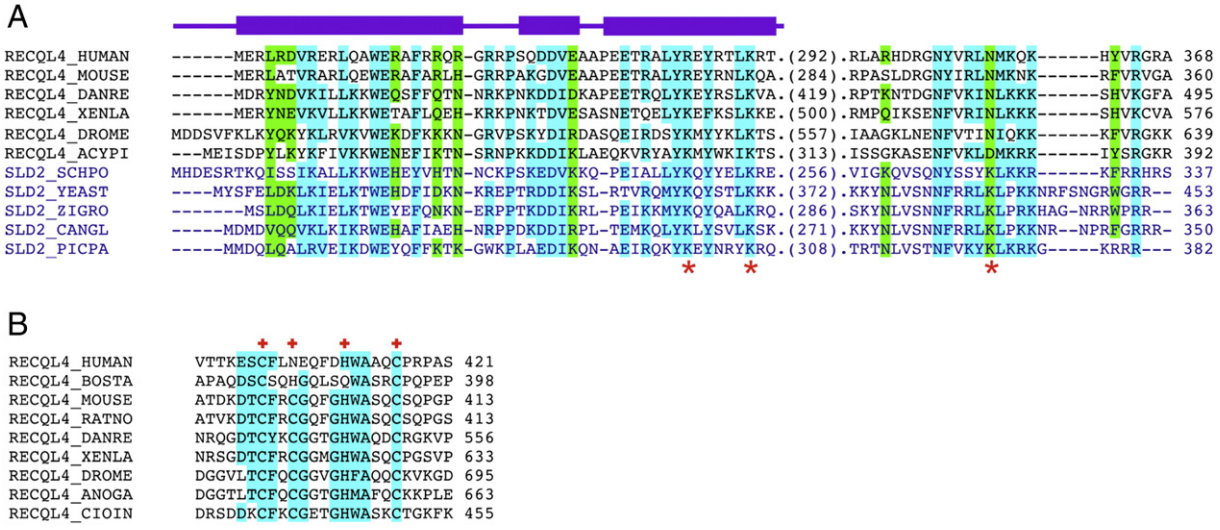

Fig. 2. The N-terminal region. A) Sequence alignment between the N-terminal domain from a selection of RecQ4 proteins and the fungi Sld2 replication factors, showing that the homology extends to the C-terminus of Sld2. In blue are highlighted the residues that are identical or very similar (such as E/D, R/K/H, V/I/L/M, G/A, Y/F/W, S/T/P, N/Q) and in green residues that show a similar character (large polar/charged, hydrophobic, aromatic, etc.). The α-helices, as revealed by the NMR structure of the first 50 amino-acid fragment of human RecQ4 (PDB ID: 2KMU) are shown as rods above the sequence. The three residues that have been implicated in binding to origin DNA for *S. cerevisiae* Sld2 [36] are highlighted by a red star. B) Sequence alignment between the N-terminal region from a selection of RecQ4 proteins, showing the putative Zn knuckle. The residues predicted to coordinate a Zn atom are identified by a red cross. In the human sequence the second cysteine is substituted by an asparagine.

While the first two helices of the Zn domain are well conserved, a long insertion is present between the second and third helix; an additional insertion is found between the end of the Zn domain and the following winged helix domain. The pattern of four cysteines coordinating a Zn atom in the atomic structure of *E. coli* RecQ [6] and human RecQ1 [7] is partially conserved. A second pattern of three cysteines and one histidine conserved in all the RecQ4 sequences can be found in the long insertion between the second and third helix, suggesting the presence of an additional Zn binding motif (Fig. 3).

A significant degree of similarity can also be found within the winged helix domain (Fig. 3). No strong signal can be identified with reasonable certainty by automatic algorithms, with the exception of a short region between 986 and 1030 that is predicted by HHpred [34] to fold as the fragment of a winged helix fold, consistent with our alignment. When the atomic coordinates of the RecQ WH domains whose structures are known (PDB IDs: 1OYW, 2V1X, 3AAF) are overlapped, a pattern of similarity emerges, including the conservation of a hydrophobic core and a key arginine involved in DNA binding [12]. Most of these residues are also conserved in RecQ4 proteins (Fig. 3).

Moreover, the WH domains of RecQ1 and WRN include a β-hairpin with an aromatic residue at the tip (Tyr564 in hRecQ1 and Phe1037 in hWRN) shown to be involved in DNA binding and unwinding, by acting as a pin to disrupt Watson–Crick base pairing [12,13,7]. In RecQ4 the β-hairpin is also conserved and the tip residue is a phenylalanine in most sequences, most likely having a similar functional role.

Despite the similarities between the canonical RQC domains and the putative RQC we suggest in RecQ4 proteins, two long insertions within the Zn domain make this region enough divergent from the canonical fold to escape detection by automatic algorithms. Further evidence of the presence of the RQC domain and the consistency of the alignment presented in Fig. 3 is provided by the conservation of residues at the interface between the helicase and the Zn domain (Table 1).

4. Discussion

The similarity between yeast Sld2 replication factors and RecQ4 reported in the literature [17] is limited to the first 150 amino-acid residues. However many evidences suggest that the essential function of the N-terminal region of RecQ4 encompasses a much larger region. Mutations causing genetic defects involve only the helicase or downstream region, suggesting that the N-terminal domain has an essential function [28]. Knock-out mice lacking exons 5–8 result in embryonic lethality [39]. Moreover, cells where RecQ4 expression is turned off can be rescued by the expression of the N-terminal region (1-496) but not by shorter fragments [40]. The fact that we have detected an additional region of sequence similarity between the C-termini of Sld2 and the RecQ4 region located between 345 and 370 in the human sequence, suggesting that the homology encompasses the entire Sld2 protein, is coherent with the above information.

The presence of a region of significant homology corresponding to the C-terminus of yeast Sld2 implies the occurrence of a fusion event between the entire Sld2 and a RecQ helicase. Blast searches using

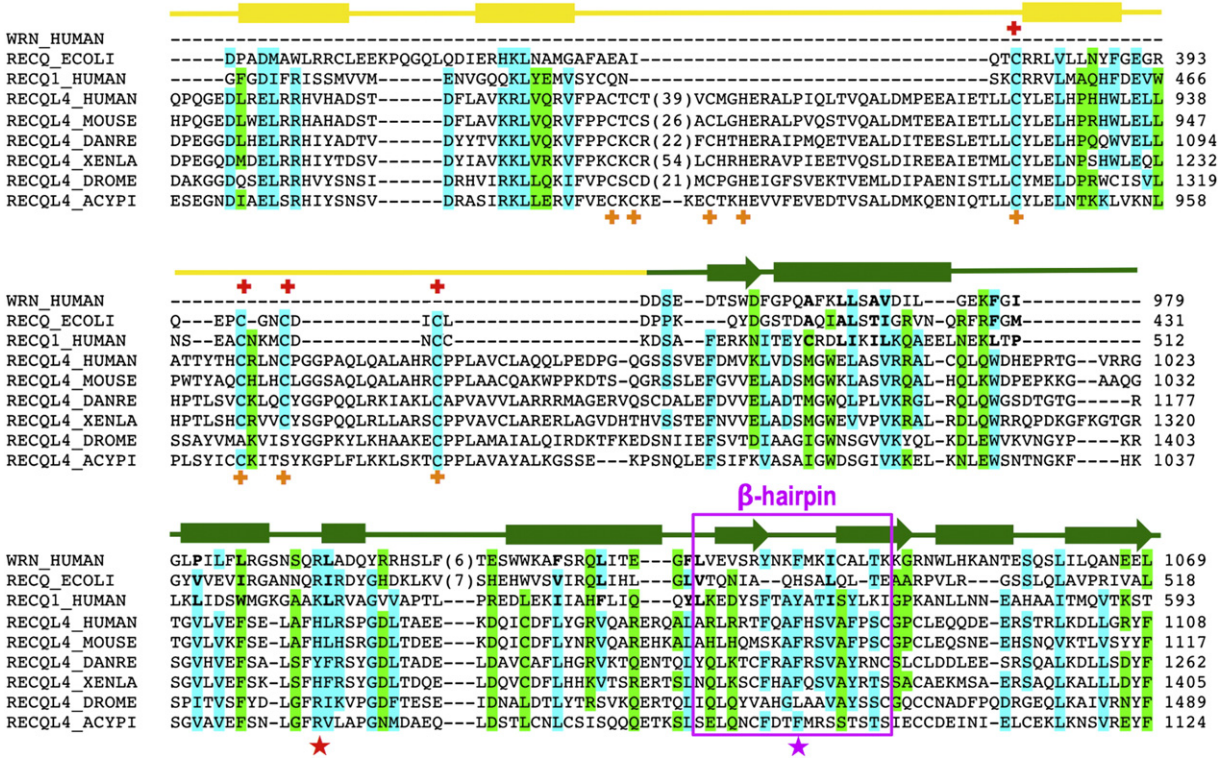

Fig. 3. The RQC domain. The putative RQC domain of RecQ4 is aligned with the equivalent region in human RecQ1 and *E. coli* RecQ, and with the WH domain of Werner. The alignment between RECQ1_HUMAN, RECQ_ECOLI and WRN_HUMAN is structure-based (PDB IDs: 1OYW, 2V1X, 3AAF). Highlighted are the residues that are conserved within the RecQ4 sequences and at least one other sequence. In blue are highlighted the residues that are identical or very similar (E/D, R/K/H, V/I/L/M, G/A, Y/F/W, S/T/P, N/Q) and in green residues that show a similar character (large polar/charged, hydrophobic, aromatic, etc.). The secondary structure elements are based on the crystal structure of human RecQ1 (α-helices are shown as rods and β-strands as arrows): in yellow is shown the region corresponding to the Zn domain, in green that corresponding to the winged helix domain. The four cysteine residues coordinating a Zn atom in the two crystal structure are shown by red crosses above the sequences; conserved cysteines which could possibly be involved in Zn binding in RecQ4 are shown by orange crosses below. The non-polar residues that have been described as part of the WH core in WRN [12] are shown in bold. The conserved positively charged residue implicated in DNA binding [12] is identified by a red star. The β-hairpin that in the RecQ1 and Werner proteins has been shown to act as a pin [7,12] is enclosed in a magenta box, with the key tyrosine/phenylalanine identified by a magenta star.

Table 1
Conserved interface between helicase and RQC domains.

	Human RecQ1	Human RecQ4	*E. coli* RecQ
Polar interactions			
$Asp326^{hRecQ1}$/$Arg425^{hRecQ1}$	Asp326	Asp712 (Asp/Glu)	
	Arg425	Arg830 (Arg)	
$Glu396^{hRecQ1}$/$His461^{hRecQ1}$	Glu396	Glu796 (Glu)	Glu318
	His461	His933 (His/Arg)	
$Asp422^{hRecQ1}$/$His461^{hRecQ1}$	Asp422	Glu827 (Glu)	Asp344
$Glu433^{hRecQ1}$/$Lys439^{hRecQ1}$	Glu433	Asp837 (Asp)	Glu318
	Lys439	Arg844 (Arg)	Lys367
$Glu433^{hRecQ1}$/$Lys393^{hRecQ1}$	Lys393	Pro793 (Pro/Arg)	Arg315
Hydrophobic interactions			
Cluster 1:	Met391	Leu791 (Leu/Met)	Ile313
$Met391^{hRecQ1}$, $Leu440^{hRecQ1}$, $Val444^{hRecQ1}$, $Ile423^{hRecQ1}$, $Tyr418^{hRecQ1}$	Tyr418	Leu818 (Leu)	Tyr340
	Ile423	Leu828 (Leu)	Met345
	Val444	Val849 (Val/Ile)	
Cluster 2:	Leu440	Leu845 (Leu)	Leu368
$Tyr399^{hRecQ1}$, $Phe462^{hRecQ1}$	Tyr399	Val799 (Val)	Tyr321
	Phe462	Trp934 (Trp)	Phe390

Further evidence of the presence of the RQC domain and the overall consistency of the alignment presented in Fig. 3 is provided by the conservation of the residues at the interface between the helicase domain and the Zn domain of the RQC. The table reports the conserved residues at the interfaces. Between parentheses are shown the residues common to the majority of the RecQ4 orthologues. Salt bridges and polar interactions found in the crystal structure of human RecQ1 [7] and/or *E. coli* RecQ [6] are conserved (or partially conserved); substantially conserved are also the hydrophobic interfaces.

various Sld2 homologues as targets identified the presence of an isolated Sld2 in Fungi, and of a fusion protein in Metazoa, Choanoflagellata and Viridiplantae. This fusion event strongly argues in favour of a role for RecQ helicases in the early processes of eukaryotic DNA replication [25].

We have recognised an additional conserved feature in the N-terminal region, likely to fold into a Zn knuckle. Whereas most of the sequences possess the canonical CCHC pattern of Zn^{2+} ligands, in the human sequence the second histidine is substituted by an asparagine (CNHC), and in the bovine sequence the histidine is substituted by a glutamine residue (CCQC). Amino acids such as asparagine and glutamine are uncommon Zn^{2+} ligands, but have been occasionally seen [41,42].

This domain is located in the region that appears essential for cell viability and is likely to share with the Sld2-homology domain the essential function of RecQ4 in DNA replication. We cannot yet speculate on the exact role of the Zn knuckle: although it may simply have a structural purpose, it could also be involved in nucleic acid binding. Indeed a variety of Zn knuckles have been shown to bind both ssDNA and ssRNA. Most have a preference for ssRNA binding [37,38], but the module has also been found in transcriptional activators binding to promoter DNA [43] and cellular nucleic-acid binding proteins involved in ssDNA binding [44]. Another possibility is that the Zn knuckle may be involved in protein–protein interactions: it is interesting to note that the region 361 to 478, encompassing the Zn knuckle, has been implicated in the interaction with Bloom helicase [45].

Studies on RecQ4 helicases have long been hindered by initial reports suggesting that they did not possess any DNA helicase activity, despite the presence of all the canonical helicase motifs found in the SF2 helicase family; indeed the absence of a RQC domain was generally proposed as an explanation for the lack of helicase activity. In recent years, various laboratories have demonstrated that the recombinant protein is an active DNA helicase [14,8,15,16]; in this context the absence of a RQC was rather puzzling, as both biochemical and structural data suggested a crucial contribution to the RecQ helicase activity. Since the RQC is part of the RecQ catalytic core and is essential for the activity [12,13,7], our proposal that RecQ4 proteins do indeed possess a RQC domain is consistent with the more recent biochemical results. Interestingly, several RecQ4 patient mutations [28] reside in the newly identified RQC domain, pointing to an important role of the RQC in the regulation of protein activity.

5. Conclusions

Of the three RecQ that cause genetic diseases in humans (Bloom, Werner and RecQ4 helicases), RecQ4 is the least well known. We have carried out a detailed bioinformatic analysis of RecQ4 proteins that has revealed the presence of a number of features that have not been previously described, including an extension of the Sld2-homology region, a novel Zn knuckle upstream of the helicase domain and, most notably, a putative RQC domain following the helicase core. The presence of a RQC domain, which in other RecQ proteins has been shown to be involved in the helicase mechanism, is consistent with recent reports demonstrating that RecQ4 proteins are indeed able to catalyse the unwinding of dsDNA.

This analysis provides significant insights for the elucidation of the architecture and the cellular roles of these poorly characterised proteins. This is a pre-requisite to unravel the molecular basis of the Rothmund-Thomson syndrome and other related diseases caused by mutations in the *RECQ4* gene and to correlate the mutational spectrum observed in patients to the structure and function of the protein.

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