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RecQ and Fe–S helicases have unique roles in DNA metabolism dictated by their unwinding directionality, substrate specificity, and protein interactions

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Abstract

Helicases are molecular motors that play central roles in nucleic acid metabolism. Mutations in genes encoding DNA helicases of the RecQ and iron–sulfur (Fe–S) helicase families are linked to hereditary disorders characterized by chromosomal instabilities, highlighting the importance of these enzymes. Moreover, mono-allelic RecQ and Fe–S helicase mutations are associated with a broad spectrum of cancers. This review will discuss and contrast the specialized molecular functions and biological roles of RecQ and Fe–S helicases in DNA repair, the replication stress response, and the regulation of gene expression, laying a foundation for continued research in these important areas of study.

Conserved helicase domains and structural aspects

Conserved amino acid sequences (or motifs) comprising the two conserved RecA-like motor domains within the ATPase core domain have been used to categorize RecQ and Fe–S helicases into two families within the superfamily (SF) 2 grouping [1] (Figure 1). RecQ family members (with the exception of RECQL4 [2] and RECQL5 [3]) also possess a RecQ C-terminal (RQC) region consisting of Zn²⁺-binding and winged helix (WH) domains, and (in WRN and BLM) a helicase RNase D-like C-terminal (HRDC) domain [2–6] (Table 1), both important in nucleic acid interaction/substrate specificity [7–9] and protein interaction, particularly in WRN [10–14]. While RECQL4 also harbors a Zn²⁺-binding domain, it is unique and coordination of the metal ion is distinct from that of the RQC domain found in other RecQ helicases [2]. RECQL5, on the other hand, harbors a Zn²⁺-binding domain with structural similarity to other RecQ helicases, flanked by an α helix with surface-exposed positively charged residues thought to be analogous to the β -hairpin found in other RecQ helicases that serves as a wedge for DNA duplex separation [3] (Table 1). WRN and RECQL4 are unique in that they harbor proof-reading exonuclease [15] and replication

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initiator SLD2 [16] domains, respectively (Figure 1). RECQL5 interacts with RNA polymerase II via a kinase-inducible (KIX) domain in its C-terminus [17] (Figure 1).

For Fe–S helicases, the Fe-chelating cluster and Arch domains residing within the helicase core are a hallmark of that family [18,19] (Figure 1), and several Fe–S XPD helicase structures with distinct features have been reported [20–24]. Based on these structural data and biochemical studies [25], the Fe–S and Arch domains together are proposed to serve as a wedge for duplex DNA unwinding. FANCI and RTEL1 are unique in that they harbor interaction domains for the tumor suppressor BRCA1 [26] and DNA clamp proliferating cell nuclear antigen (PCNA) [27], respectively (Figure 1). Mapping studies have identified several other regions in FANCI that mediate protein interactions [28]. An overarching distinction between RecQ and Fe–S helicases is their directionality of DNA translocation used for unwinding (discussed below), which in Fe–S cluster helicases has been experimentally elucidated by structural and biochemical studies [22,29] (see below).

DNA unwinding mechanisms

It is apparent from structural and biochemical studies of SF2 DNA helicases that the RecA motor core binds and hydrolyzes a nucleoside triphosphate (typically ATP) in a manner that is allosterically activated by single-stranded DNA binding and the free energy is transduced for the helicase to translocate on DNA and disrupt hydrogen bonds between complementary A–T and G–C base pairs of the two strands [30]. Although the conserved motifs within the two RecA motor domains and their unique arrangement are likely to control the 3′–5′ polarity of translocation by RecQ helicases [31], it is still unclear the precise mechanism by which this unidirectional movement is achieved. It is believed based on structural analysis of helicase–DNA complexes (e.g. RECQL1 [6]; *Thermoplasma acidophilum* XPD [22]) and biochemical studies with DNA substrates that contain modifications to the sugar-phosphate backbone [32–34] that both the RecQ and Fe–S helicases predominantly interact with the negatively charged phosphate backbone. However, protein contacts with the bases themselves are also important as evidenced by helicase assay data with DNA substrates containing base lesions [35–38]. For example, both RECQL1 and FANCI helicases are inhibited by thymine glycol residing within the duplex region in either the translocating or non-translocating strand [38], suggesting that these helicases operate by an active mechanism in which they interact with both DNA strands during duplex DNA unwinding. Lesion- and strand-specific differences in the interactions of SF2 helicases with damaged DNA substrates are also apparent, lending support to the idea that subtle distinctions between mechanisms of DNA unwinding by helicases of the RecQ and Fe–S families are likely and that DNA damage may affect helicase function in unique ways [39,40].

The conserved WH, originally identified in the crystal structure of *Escherichia coli* RecQ [41], harbors a β -hairpin in RECQL1 that is essential for DNA-strand separation and mediates dimer formation [42,43] (Table 1). RECQL1 exists in multiple assembly states with monomers and dimers responsible for duplex unwinding and tetramers capable of HJ (Holliday Junction) resolution. The dimer interface helps to position the strand-separating β -hairpin for optimal helicase activity, whereas the planar tetramer plays a critical role in HJ recognition [6]. For the RecQ helicases, a conserved aromatic-rich loop (ARL) residing

within one of the two RecA domains couples single-stranded DNA binding to ATP hydrolysis, which is required for strand separation during duplex DNA unwinding [2,43–45] (Table 1). Mutation of conserved residues in the ARL of RECQL1 also inactivates its ATP hydrolysis-dependent DNA branch migration, but not its strand annealing activity which is independent of ATP hydrolysis [44].

The structure of a BLM–DNA complex reveals three contiguous bases pointing in an opposite direction to the preceding ones, suggesting a novel base-flipping mechanism [5]. Perhaps, one of the most striking findings from the BLM structure is that the HRDC domain is positioned closely to a cleft between the two RecA domains where the bound ADP resides, suggesting a prominent role in ATP hydrolysis.

Although RECQL4 lacks a WH domain, the C-terminal region of the protein is critical for helicase activity [2]. Nonetheless, the apparent absence of a prominent wedge structure in RECQL4 may underlie its limited processive duplex unwinding; however, a RECQL4–DNA complex is required to draw further conclusions about its mechanism of DNA unwinding.

For the Fe–S helicases, the original crystal structures solved are of different species of the thermophile XPD [18,20,40] and implicate the conserved Fe–S and Arch domains (Figure 1) in duplex DNA separation. The co-crystal structure of *Thermoplasma acidophilum* XPD with a short (22-mer) single-stranded DNA oligo-nucleotide revealed the path of the translocated strand through the helicase pore [22]. Based on a comparison of the XPD structure with those of helicases with opposite polarities (3′–5′), it was inferred that the orientations of the bound DNA molecules by the two RecA domains were the same, suggesting that directionality of translocation is achieved by helicase protein gripping of the single-stranded DNA in a reverse manner. Another study which mapped XPD protein domain contacts on DNA agreed with the structural studies [29], and together the findings suggested that 5′–3′ polarity of movement by XPD on single-stranded DNA is achieved by orientation-specific conformational changes within the motor domain.

Of the Fe–S helicases, only FANCF has been reported to oligomerize [46]. The dimeric form of FANCF was observed to display increased DNA binding compared with the monomeric form, as well as a significantly greater specific activity for ATP hydrolysis and duplex DNA unwinding. Because mutation of the conserved Q motif in FANCF was shown to negatively affect its dimerization [46], it will be interesting to assess if this region affects oligomerization or protein interactions of other RecQ and Fe–S helicases.

Because both the RecQ and Fe–S helicases share the same RecA motor core, it is plausible that their auxiliary domains not only contribute to DNA translocation directionality, but also influence nucleic acid substrate specificity and protein interactions. Clearly, there are unique functions of these helicases, as evidenced by their biochemical activities and protein partners, as well as the distinctive clinical features and chromosomal instability typical of the genetic deficiencies.

Clinical features and chromosomal abnormalities

Hereditary diseases are linked to mutations in three of the five RecQ helicase genes; however, deficiency in any of the human RecQ helicases results in chromosomal instability (Table 2). Recessive mutations in RecQ helicase genes *BLM* and *WRN* give rise to Bloom's syndrome (BS) and Werner syndrome (WS), respectively [47,48], with distinct forms of chromosomal instability. Persons with BS display short stature, photosensitivity, immune deficiency, and predisposition to a broad range of cancers [49]. WS is characterized by premature aging, punctuated by early onset of Type II diabetes, osteoporosis, cardiovascular diseases, cataracts, and sarcomas [50]. Mutations in *RECQL4* result in three separate syndromes with largely overlapping phenotypes: Rothmund-Thomson syndrome (RTS), Baller-Gerold syndrome (BGS), and RAPADILINO syndrome [51]. The characteristic clinical features may be influenced by environmental factors and display a broad range in severity; all three include stunted growth, radial ray defects, and skeletal abnormalities [52]. The main and characteristic diagnosis for RTS is poikiloderma. In addition, RTS patients often display cataracts and early hair loss, whereas BGS is associated with craniosynostosis [52]. Patients with RAPADILINO syndrome suffer from diarrhea and joint dislocation in addition to the overlapping phenotypes. In terms of *RECQL4* mutations and cancer susceptibility, individuals with RTS are at a higher risk for osteosarcoma (primarily bone cancers) as well as skin cancers (basal and squamous cell carcinoma). RAPADILINO patients are at a higher risk of developing osteosarcoma and lymphoma [51,52]. Mutations in *RECQL1* and *RECQL5* have not been linked to genetic diseases, but *RECQL1* mutations are associated with increased breast cancer susceptibility [53,54].

Chromosomal instability is also caused by defective Fe-S helicases (Table 2). *FANCI* (also designated *BRIP1* or *BACH1*) encodes an Fe-S DNA helicase and is one of over 20 genes in which mutations are responsible for Fanconi Anemia (FA) [55], characterized by progressive bone marrow failure (BMF) [56], cancer, and congenital abnormalities [57]. Recent evidence suggests that FA phenotypes are due to an inability to repair cellular DNA damage induced by endogenous formaldehydes, leading to failure of the hematopoietic stem cell compartment [58]. Bi-allelic mutations in *DDX11* are linked to Warsaw Breakage syndrome (WABS), a cohesinopathy also characterized by chromosomal breakage induced by the cross-linking agent mitomycin C (MMC), similar to FA [59]. Mutations in *RTEL1* are linked to Dyskeratosis congenita (DC) and the more clinically severe disease variant Hoyeraal-Hreidarsson syndrome (HHS) [60] (Table 2). Principal features of DC and HHS are believed to arise from telomere instability and include BMF, reticulated skin hyperpigmentation, nail dystrophy, oral leukoplakia, and microcephaly. Mutations in *XPD*, which encodes an Fe-S helicase of the TFIIH complex implicated in transcription and nucleotide excision repair (NER), are linked to three disorders: Xeroderma pigmentosum (XP), Cockayne syndrome (CS), and Trichothiodystrophy (TTD), or combined XP/CS and XP/TTD [61,62]. XP is characterized by extreme sunlight sensitivity, multiple skin abnormalities, and skin cancer. CS individuals are solar-sensitive and also intellectually challenged and display dwarfism, microcephaly, and retinal/skeletal abnormalities. TTD individuals are intellectually challenged, but only some are sunlight-sensitive. Remarkably, cancer is absent in TTD or CS

[63]. Genotype–phenotype relationships of *XPD* mutations and their relevance to clinical symptoms and cancer are of great interest.

Biochemistry

A fundamental difference between RecQ (3′–5′) [87–91] and Fe–S (5′–3′) [92–95] helicases is their directionality of single-stranded DNA translocation (fueled by nucleoside triphosphate binding/hydrolysis) used for unwinding a duplex DNA substrate. Helicase directionality is appreciated conceptually in the context of DNA synthesis during replication or repair. RecQ helicases that translocate 3′–5′ along the same template strand used by a DNA polymerase (pol) may be better equipped to remove unusual DNA structures during replication or DNA repair synthesis. Indeed, several human RecQ helicases (e.g. WRN) interact physically and functionally with DNA pols (*Protein Interactions*); however, FANCI also functions to overcome roadblocks during replication (see below).

Helicases of both the RecQ (WRN [96], BLM [97], and RECQL1 [98]) and Fe–S (FANCI [95], DDX11 [99], and *Ferroplasma acidarmanus* XPD [25]) families preferentially unwind forked duplex substrates compared to substrates with only one single-stranded DNA extension. This is generally attributed to preferential substrate binding; however, there is apparent complexity in DNA substrate specificity and mechanism of loading as several RecQ helicases (WRN [96], BLM [100,101], and RECQL1 [98]) quite efficiently unwind 5′-flap DNA substrates (that lack any pre-existing 3′-single-stranded DNA tail). Biochemical studies using biotinylated DNA substrates with streptavidin bound at specific sites suggest that WRN recognizes the DNA junction and loads accordingly to perform unwinding of the parental forked duplex [96], which may be relevant to its role in lagging strand processing [64] (Figure 2) or strand displacement synthesis during base excision repair (BER) [102]. The Fe–S helicases FANCI [95] and DDX11 [99] also robustly unwind 5′-flap substrates, presumably by traditional tracking along the 5′-single-stranded arm (Figure 2), although fork recognition may be relevant. In yeast, sister chromatid cohesion (which involves the ortholog of human ChlR1/DDX11 [83,84]) is coupled with lagging strand processing [103,104] and involves Ctf4 recruitment of Chl1 to the replisome [105], suggesting that DDX11 interactions with replisome-associated proteins [e.g. Flap Endonuclease 1 (FEN-1) [94] and Timeless [106]] are important (Table 3 and Figure 2). *T. acidophilum* XPD was shown to unwind a forked duplex, but to our knowledge its preference for that substrate compared with other partial duplex DNA molecules was not evaluated [22]. *Sulfolobus acidocaldarius* XPD was reported to unwind a forked DNA duplex with similar efficiency to the same duplex flanked by only one single-strand, but the helicase can also unwind a bubble substrate lacking free ends, a key intermediate of NER [107].

RecQ and Fe–S helicases are further distinguished by their substrate specificity. Helicases of both families are implicated in homologous recombination (HR) repair of double-strand breaks (DSBs), suggesting that they catalytically resolve recombinant DNA molecules [e.g. three-stranded displacement (D)-loop or four-stranded HJ] to help mature the multi-stranded intermediates or prevent illegitimate recombination between non-homologous duplexes (Figure 2), thereby suppressing genomic instability and mutations. Indeed, RecQ (e.g. WRN

[119,120], BLM [121], RECQL1 [98], and RECQL5 [122]) and Fe-S (e.g. FANCF [95], DDX11 [99], and RTEL1 [123]) helicases unwind fixed D-loops (Figure 2); furthermore, human RecQ enzymes branch-migrate mobile D-loops [119,124,125]. However, while WRN [126], BLM [97], RECQL1 [98,127], and RECQL5 [88] preferentially resolve HJs *in vitro*, FANCF [95] and DDX11 [99] fail to do so and there is no report for RTEL1 or XPD. Thus, certain RecQ helicases may have specialized duties to resolve HJs during late-stage DNA recombination events or replication fork remodeling. Although many RecQ helicases catalyze strand annealing of complementary single-stranded DNA sequences [128], the biological significance of this activity remains to be determined.

RNA (R)-loops that form when a stable RNA–DNA duplex persists during transcription can cause replicational stress and interfere with normal genome homeostasis [129]. Motor ATPases/helicases such as Pif1 [130] or Sen1 [131] in yeast or human FANCD1 [132] may help to suppress transcription–replication conflicts by resolving RNA–DNA hybrids and/or displacing the transcription protein complex. BLM (but not RECQL1) [133], WRN [134], and FANCF [92] were reported to unwind RNA–DNA duplex substrates; however, cellular evidence for their role in R-loop metabolism is lacking. DEAD-box RNA helicases such as DDX21 [135] may primarily resolve R-loops. RECQL5, which directly interacts with RNA polymerase II [136–138], prevents transcription-associated genomic instability by acting as a regulatory transcription elongation factor [71] or allowing RNA polymerase passage [139] (Figure 2).

G-quadruplex (G4) DNA may play a role in R-loop metabolism and can affect telomere stability, gene regulation, replication initiation and progression, and hotspot genetic recombination [140,141]. Certain RecQ helicases (e.g. WRN [97,142], BLM [143], and RECQL5 [144]) and Fe-S helicases (e.g. FANCF [145,146], RTEL1 [27], DDX11 [99], and *S. acidocaldarius* XPD [147]) unwind G4 DNA substrates *in vitro*, which is likely to be relevant to their specialized roles during replication (Figure 2). Of the Fe-S helicases, only FANCF was shown to preferentially unwind entropically favored unimolecular G4 DNA substrates [148]. Recently, a G4-specific recognition site was mapped in FANCF [149]. FANCF and its orthologs play an important role in G4 DNA resolution to enable smooth DNA synthesis during replication [145,146,150,151]. FANCF and WRN/BLM or the translesion polymerase REV1 may co-ordinate in leading and/or lagging strand replication (Figure 3). Moreover, FANCF's role in G4 resolution affects chromatin compaction and recycling of histones that influences heterochromatin assembly and gene expression [152,153]. In human cells, FANCF also acts to suppress DSBs by stabilizing genome-wide microsatellite repeats prone to form hairpin structures which stall replication forks [73,76].

In addition to replication, G4-interacting helicases influence gene expression. This was first suggested by microarray analyses of gene expression patterns in fibroblasts from individuals with WS or BS [154]. Expression profiling of messenger RNA and microRNA from BS cells or BLM-depleted cells by RNA interference suggested that BLM regulated expression of genes with enriched G4 motifs at the transcription start site or exon 1/intron 1 boundary [155]. WRN is also likely to regulate expression of genes with enriched G4 motifs at these locations, only in a different set compared with BLM, suggesting distinct roles of the two RecQ helicases in disease pathogenesis [156]. Other studies that included chromatin

immunoprecipitation experiments suggested that RECQL1 [157], XPB, and the Fe–S helicase XPD [147] regulate gene expression directly through their interactions with predicted G4-forming sequences in promoters. Certain RecQ and Fe–S helicases also preferentially interact with and resolve unusual DNA structures in the G-rich telomeric repeats (see *Telomeres*) (Figure 2).

Protein interactions and genome maintenance pathways

Protein interactions play an important role in the functions of RecQ [158,159] and Fe–S helicases (Table 3). The single-stranded DNA-binding protein Replication Protein A (RPA) is a partner of both RecQ and Fe–S helicases that greatly enhances DNA unwinding by these slowly processive helicases [160]. Protein mapping and biochemical studies demonstrated that the physical interaction of WRN with RPA is required for stimulation of helicase-catalyzed DNA unwinding activity [161]; this is probably true for the other RecQ and Fe–S helicases based on observations that heterologous single-stranded binding proteins poorly stimulate their unwinding activities.

BLM resides in a protein complex with topoisomerase (Top)III α , RMI1, and RMI2 that is believed to dissolve double HJ structures during HR repair or replication fork convergences to prevent elevated sister chromatid exchange (SCE) [162] (Figure 2), a potential source of chromosomal instability and a clinical marker for BS [49] (Table 2).

WRN interacts with several DNA pols (pol δ [163–165] and translesion DNA pols [12,166]) to stimulate DNA synthesis and improve fidelity via WRN's 3'–5' exonuclease activity. WRN is recruited to stalled replication forks to aid in fork recovery and restart [126,167–172]. A prominent partner of WRN is FEN-1 that cleaves 5' flaps during BER and lagging strand DNA synthesis [10,173] (Figure 2). WRN co-ordinately stimulates pol δ strand displacement synthesis and FEN-1 cleavage of 5' flaps [174] (Figure 3). Like WRN, FEN-1 deficiency diminishes lagging strand telomeric DNA replication; moreover, expression of a FEN-1 mutant defective in its interaction with WRN and shelterin protein TRF2 failed to rescue the telomere defect [175]. BLM also interacts with FEN-1 through a non-catalytic domain conserved in WRN [101]. Both WRN and BLM interact with TRF1 and TRF2 to preserve the normal lengths and stability of telomeres [176–178], but the mechanism(s) is still not well understood.

In *Xenopus*, initiation of DNA replication requires RECQL4 [16]. Subsequently, it was found that in human cell extracts, RECQL4 interacts with the replicative MCM helicase complex and associated factors [179], and the helicase is recruited to replication origins where it regulates proliferation by controlling initiation of DNA synthesis [180].

FANCF was originally discovered by its interaction with BRCA1, and disruption of their interaction compromises DSB repair by diminishing HR healing of ionizing radiation-induced chromosome breaks [26]. FANCF is implicated in interstrand cross-link (ICL) repair, and interaction with the mismatch repair (MMR) protein MLH1 is critical for suppressing MSH2's engagement in a deleterious DNA damage response [111,112], but the molecular mechanism remains to be elucidated. FANCF also interacts with BLM, and the

two helicases synergistically unwind a damaged DNA substrate [113]. FANCI's interaction with BLM (which influences BLM stability) is likely to contribute to the elevated SCE and poor replication stress response in FANCI-deficient cells [113] (Table 2).

A key partner of RTEL1 is the DNA synthesis processivity factor PCNA, which facilitates RTEL1's association with the replication machinery and promotion of genomic DNA replication and telomere stability [27] (Table 2 and Figure 3).

RECQL4 and XPD are the only known RecQ and Fe-S helicases, respectively, to localize to mitochondria. XPD plays an important role in the repair of oxidized base lesions that form in the organelle's unique environment of reactive oxygen species [181]. RECQL4 is thought to play a role in mitochondrial replication [182–184], supported by its stimulatory interaction with mitochondrial DNA pol γ [185] (Figure 2). Model systems are required to further characterize the roles of RECQL4 and XPD in mitochondrial genome metabolism *in vivo*.

Replication fork remodeling and DNA repair mechanisms

Although BLM [186,187], WRN [187], and RECQL5 [137] catalyze fork regression *in vitro* (Figure 2), *in vivo* evidence that this activity is required to stabilize stalled replication forks is lacking. DNA fiber analyses suggest that WRN helicase aids the helicase–nuclease DNA2 to degrade reversed replication forks to allow fork restart and prevent fork processing events detrimental to genomic stability [172]. To date, none of the Fe-S helicases have been shown to catalyze fork regression or fork remodeling, even *in vitro*. It seems that the FBH1 helicase [188] or SMARCA1 translocase [189] might be better suited to act in this capacity. Biochemical and cellular evidence suggests that RECQL1 restores regressed forks to structures capable of restarting DNA synthesis by catalyzing reverse branch migration of the HJ structure in a manner that is regulated by the DNA damage sensor poly(ADP)ribose polymerase (PARP) 1 [190] (Figure 3). RECQL1 also governs the pool of RPA during replication stress to maintain normal fork progression and suppress DNA damage [44].

The Fe-S helicases XPD and FANCI are implicated in NER and ICL repair, respectively. Biochemical analysis of site-directed NER-defective XPD mutants demonstrated that the corresponding amino acid substitutions in *F. acidarmanus* XPD compromise the enzyme's ability to be inhibited or sequestered by a cyclobutane pyrimidine dimer (CPD) lesion [191], suggesting that failure to form a stable protein–DNA complex is responsible for the NER defect. In another study, biochemical evidence showed that the NER machinery, like XPD helicase, translocates 5'–3' to enact removal of a CPD *in vitro* [192]. Together, the findings suggest (but do not prove) that XPD plays a role in NER damage recognition by facilitating a scanning mechanism to verify the lesion (Figure 2). Ultimately, this damage verification by XPD would set the stage for incisions and removal of the damaged oligonucleotide followed by DNA repair synthesis (for review, see ref. [193]). Consistent with this model, XPD's ATPase/helicase function is instrumental for its NER function [21]. Experimental evidence demonstrates that p44, a subunit of TFIIH, interacts with XPD (Table 2 and Figure 1) and stimulates its helicase activity [93]. Furthermore, it is proposed that XPD positions the cyclin-dependent kinase-activating kinase (CAK) complex for optimal transcription and

CAK, in turn, represses XPD helicase activity to regulate TFIIH during the damage verification step of NER [117].

FANCI is believed to operate in downstream events of the FA ICL repair pathway to facilitate HR repair of the broken replication fork (i.e. DSB) arising from the unhooking step enacted by ICL nucleolytic incisions [194]; however, FANCI's *in vivo* molecular mechanism of action is still poorly understood. FANCI-deficient cells were shown to display reduced HR repair in a cell reporter assay [194], consistent with its BRCA1 interaction [26]. The involvement of FANCI with other FA gene products [e.g. BRCA1 (FANCS), RAD51 (FANCR), RAD51C (FANCO), PALB2 (FANCN), and BRCA2 (FANCD1)] in HR repair is still under intense investigation. *In vitro*, FANCI can displace the major strand exchange protein Rad51 bound to DNA [195] (Figure 4), suggesting that it may act as an antirecombinase or help to mature recombinant DNA molecules for DNA synthesis and ligation. The sequence-related RTEL1 helicase is also proposed to regulate HR repair by acting upon D-loop (displacement loop) intermediates (Figure 4), though it is incapable of displacing Rad51 bound to single-stranded DNA [123]. Fe-S or RecQ helicases may collaborate with MMR proteins to suppress inadvertent HR by D-loop dissociation of imperfectly paired DNA sequences.

DSB repair by a HR pathway in replicating cells requires extensive 5'-strand resection to generate 3' single-stranded tails for Rad51-mediated invasion into recipient sister chromatid duplex [196]. BLM was shown to interact with the structure-specific nuclease DNA2 or EXO-1 to promote strand resection [197] (Figure 4). Roles for WRN [198] and RECQL4 [199] in strand resection and RECQL5 in synthesis-dependent strand annealing [72] were also reported, suggesting some functional overlap or parallel pathways of human RecQ helicases in DSB repair. RECQL5's efficient ability to disrupt Rad51 presynaptic filaments is proposed to suppress inappropriate HR [122] (Figure 4). Directionality of D-loop invasion may dictate the engagement of RecQ helicases (e.g. RECQL1 [125]) to suppress dead-end recombination intermediates with 5'-tail invasions that are refractory to DNA polymerase extension (Figure 4). For further reading on mechanistic aspects of recombination, see refs [200–202]. Recent data suggest that RECQL5 removes RAD51 from stalled forks to allow MUS81 structure-specific cleavage at the fork and promotes mitotic DNA synthesis at common fragile sites [203] (Figure 3).

Telomeres

The distinct 5' versus 3' single-stranded tail requirements for Fe-S helicases (e.g. FANCI [146]) versus RecQ helicases (e.g. WRN [97] and BLM [143]) to unwind adjacent G4 structures suggest that they would be positioned differently and act by distinct mechanisms at telomeric G4 DNA, as they might also in replication (Figure 4). RTEL1 [204], like WRN [64] and BLM [205,206], is implicated in telomere metabolism; however, their roles are apparently distinct. Telomere defect as an underlying basis for the accelerated aging in WS was first suggested by studies of mice deficient in both WRN and telomerase [207–209]. In human cells, WRN deficiency causes loss of telomeres specifically replicated by lagging strand synthesis [64]; telomere loss and instability are also observed in cells from HHS individuals with bi-allelic *RTEL1* mutations (Table 2). RTEL1 deficiency in mouse

embryonic fibroblasts causes G4 ligand-induced telomere fragility [204]. The consequence of BLM deficiency is debated as one study suggested a role of BLM in lagging strand telomeric DNA synthesis [206], whereas another study suggested a role of BLM in leading strand telomeric DNA synthesis [205].

Although both WRN [210] and RTEL1 [204] resolve telomeric (T) D-loops (so-called T-loops) (Figure 2), their functions are different. WRN is specifically found at telomeres of human cells that maintain their chromosome ends by an alternative lengthening of telomere (ALT) pathway independently of telomerase; moreover, WRN combines its dual exonuclease and helicase activities to resolve the T-loop (telomeric loop) in a manner that is negatively regulated by TRF1 and TRF2 [210] (Figure 4). Conversely, RTEL1 resolves T-loops solely by its ATP-dependent helicase activity [204] (Figure 4). TRF2 recruits RTEL1 to telomeres during S-phase, and by resolving the T-loop, RTEL1 suppresses T-loop cleavage by nucleases that would otherwise cause telomere fragility [116].

Molecular basis of disease

Notable differences in the molecular functions and interactions of the RecQ and Fe-S helicases must play a significant role in the unique cellular and clinical phenotypes, but the underlying mechanisms remain unclear. The distinctions may hinge on cell-type or tissue-specific expression, or complex regulation that might even depend on development, as is the case for WS where clinical features do not present robustly until the adolescent growth spurt. Demarcation of cell lineage-specific expression and operation of the RecQ and Fe-S helicase-dependent pathways may be an insightful approach to untangling the seemingly complex pathways and underlying basis for disease states.

Linking precisely helicase deficiencies to disease is enigmatic. For example, progressive BMF observed in HHS and FA is not observed in the RecQ helicase disorders such as WS. If telomere deficiency due to RTEL1 deficiency underlies the clinical features including BMF observed in HHS, then WRN's role in telomere metabolism and other DNA metabolic pathways must be distinct in its mechanism and its consequences. Flipping the coin, although FA displays some aspects of accelerated ageing [211], the disease does not show so many of the pronounced clinical features of premature aging characteristic of the RecQ disorder WS [212]. Complex regulation of helicase-dependent pathways in stem cells or during embryogenesis is likely to be important. Roles of helicases in chromatin structure and epigenetic pathways are also probably contributing to disease phenotypes. For example, studies of a WS stem cell model implicated a role of WRN in maintaining normal heterochromatin stability that aids in suppressing cellular aging, which is probably relevant to physiological aging [213]. Deficiencies in helicases that have unique functions in rapidly dividing tissues versus those with slow-turnover may dictate disease risk. In this regard, the effects of helicase deficiency may be related to telomere dysfunction in some cases, given the importance of proper telomere capping in high-turnover tissues [214].

The heterogeneity arising from mutations in even a single helicase gene linked to different diseases [e.g. *RECQL4* or *XPB* (Table 2)] reiterates the underlying complexity in defining genotype–phenotype relationships. The location of the helicase mutation (particularly

missense ones) in domains responsible for catalysis, protein interaction or regulation may also have a strong influence on disease pathogenesis [215,216].

From the literature, it is evident that defects in RecQ and Fe-S helicases clearly drive cancers; therefore, these helicases can be collectively classified as genome caretakers [158]. Conversely, expression of RecQ helicases is generally up-regulated in a spectrum of cancer types, suggesting that they are required to deal with the abundance of replicative lesions arising in rapidly dividing cells [158]. This may well be the case for the Fe-S helicases, but requires further systematic investigation. The requirement for helicase function during tumorigenesis is highly relevant to the cancer therapy field in which proteins such as helicases implicated in the DNA damage/replicational stress response or DNA repair are suggested to be potential targets in appropriate regimes [217]. The development of genetic models focusing on helicase-dependent pathways will help to advance the field toward potential translational efforts in the diagnosis, treatment, and cure of helicase disorders.

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Abbreviations

ARL	Aromatic-rich loop
BER	base excision repair
BGS	Baller–Gerold syndrome
BLM	Bloom’s syndrome helicase
BMF	bone marrow failure
BRCT	BRCA1 C-terminal
BS	Bloom’s syndrome
CAK	cyclin-dependent kinase-activating kinase
CPD	cyclobutane pyrimidine dimer
CS	Cockayne syndrome
DC	Dyskeratosis congenita
D-loop	displacement loop
DSB	double-strand break
FA	Fanconi anemia

FANCI	Fanconi Anemia Group I helicase
FEN-1	flap endonuclease 1
Fe-S	iron-sulfur
G4	G-quadruplex
HHS	Hoyeraal-Hreidarsson syndrome
HJ	Holliday Junction
HR	homologous recombination
HRDC	helicase RNase D-like C-terminal
ICL	interstrand cross-link
KIX	kinase-inducible
MCM	minichromosome maintenance protein complex
MMC	mitomycin C
MMR	mismatch repair
NER	nucleotide excision repair
PARP	poly(ADP)ribose polymerase
PCNA	proliferating cell nuclear antigen
RPA	replication protein A
RQC	RecQ C-terminal
RTEL1	regulator of telomere helicase
RTS	Rothmund-Thomson syndrome
SCE	sister chromatid exchange
SF	superfamily
T-loop	telomeric loop
TFIIH	transcription factor IIH
TRF	Telomere repeat binding factor
TTD	trichothiodystrophy
WH	winged helix
WRN	Werner syndrome helicase-nuclease
WS	Werner syndrome

XP	Xeroderma pigmentosum
XPD	xeroderma pigmentosum Group D

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Figure 1. Human DNA helicases of the RecQ and Fe-S families

Shown is an alignment of the RecQ (**A**) and Fe-S (**B**) human DNA helicases with their conserved ATPase/helicase domain consisting of two RecA folds. Auxiliary domains and selected protein interaction domains are also shown. (**A**) WRN is unique in that it has a proof-reading exonuclease domain. All the human RecQ helicases possess a Zn²⁺-binding domain, with RECQL4's being distinct from the others. The RQC domain in RECQL1, WRN, and BLM mediates DNA-binding and protein interactions. A KIX domain in RECQL5 mediates interaction with RNA polymerase II. The Sld2 domain in RECQL4 shares homology to the yeast DNA replication initiator protein Sld2. (**B**) XPD and RTEL1 interact with the p44 subunit of TFIIH and PCNA, respectively, via regions in the C-terminus. FANCI contains a C-terminal domain that upon phosphorylation at Ser-990 binds to the BRCT domain of BRCA1. See the text for details. BLM, Bloom's syndrome helicase; BRCT, BRCA1 C-terminus; FANCI, Fanconi Anemia Group J helicase; HRDC, helicase RNase D-like C-terminal; KIX, kinase-inducible; PCNA, proliferating cell nuclear antigen; RQC, RecQ C-terminal; RTEL1, regulator of telomere helicase; TFIIH, transcription factor IIH; WRN, Werner syndrome helicase-nuclease; XPD, Xeroderma pigmentosum Group D.

Figure 2. Various genomic processes are facilitated by the action of RecQ or Fe-S DNA helicases during DNA replication or repair

Replication and DNA repair are two areas which involve helicases of the human RecQ (WRN, BLM, RECQL1, RECQL4, and RECQL5) and Fe-S (FANCI, RTEL1, XPD, and DDX11) families to act on unique structures in nucleic acid metabolism. Some of the prominent DNA structural intermediates of the pathways are shown. RecQ and Fe-S helicases are implicated in the regulation of HR repair of DSBs and remodeling of stalled replication forks via their helicase or branch-migration activities on multi-stranded DNA structures. BLM is a prominent player in DNA end-resection (an early event in HR repair), as well as the dissolution of double HJs that arise during HR or replication fork convergence. Certain RecQ and Fe-S helicases are believed to resolve unusual DNA structures such as G-quadruplexes or telomeric D-loops to preserve genomic stability during replication and at chromosome ends. WRN, BLM, and DDX11 interact with the structure-specific nuclease FEN-1 that is implicated in Okazaki fragment processing. XPD, together with the DNA helicase XPB (not shown), resides in the TFIIH complex and is implicated in NER and transcription. RECQL5, via its interaction with RNA polymerase II, resolves conflicts between the replisome and the transcription complex. RECQL4 is unique among the RecQ helicases to be found in the mitochondria where it is proposed to facilitate replication of the organelle's circular genome through its interaction with mitochondrial replisome proteins. See the text for details. RNA Pol, RNA polymerase II.

Figure 3. Models for the involvement of RecQ and Fe-S helicases at replication forks
(A) WRN facilitates lagging strand synthesis and maturation by co-ordinate unwinding of 5' flaps and interacting with pol δ and FEN-1 to stimulate DNA synthesis and cleave flaps to allow ligation of Okazaki fragments, respectively. **(B)** RTEL1 interacts with the replication clamp PCNA to ensure processive DNA synthesis by pol ϵ and pol δ (not shown) and telomere stability. **(C and D)** The Fe-S helicase FANCI collaborates with the RecQ helicases WRN or BLM **(C)** or the translesion polymerase REV1 **(D)** to allow smooth DNA synthesis through G4-forming DNA sequences, which is important for normal epigenetic modifications of the nuclear genome. **(E)** RECQL5 displaces RAD51 from DNA to enable cleavage of stalled forks by the structure-specific nuclease MUS81 (anchored by the SLX4 scaffold protein), an event that is important during early mitosis to allow chromosome segregation. **(F)** RECQL1 restores the replication fork in a manner that is negatively regulated by PARP-1 to allow replication restart. See the text for details.

Homologous Recombinational Repair

Telomere Maintenance

Figure 4. Models for the involvement of RecQ and Fe-S helicases in homologous recombination repair and telomere maintenance

(A and B) RecQ (RECQL5 and BLM) and Fe-S (FANCI) helicases are proposed to regulate HR repair of DSBs by catalytically removing the major strand exchange protein RAD51 from single-stranded DNA overhangs. This may serve to suppress inappropriate recombination events or help to mature recombinant DNA molecules to allow HR to proceed. (C and D) RECQL1 and RTEL1 or FANCI act upon three-stranded D-loop structures via branch-migration or helicase activity, respectively, to suppress inadvertent HR, thereby preventing inadvertent and dead-end toxic DNA intermediates. (E) WRN or BLM promotes HR repair by branch-migrating four-stranded HJ intermediates. See the text for details. (F) The shelterin proteins TRF1 and TRF2 negatively regulates WRN's 3'-5' exonuclease activity at chromosome ends to maintain telomeric stability at the T-loop structure. (G) TRF2 recruits RTEL1 to T-loops to promote DNA unwinding to enable replication or DNA repair of telomere repeats. (H and I) WRN [and possibly BLM (not

shown)] or RTEL1 resolves telomeric G4 DNA structures to allow normal DNA synthesis or repair of telomeric G-rich DNA.

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Table 1

Structural domains of the RecQ helicases

Helicase	RecA domains	Zn ²⁺ -binding domain	Winged helix	Wedge, β -hairpin	Wedge, α -helix	Aromatic-rich loop	HRDC
RECQL1	✓	✓	✓	✓	×	✓	×
RECQL5	✓	✓	×	×	✓	✓	×
BLM	✓	✓	✓	✓	×	✓	✓
RECQL4	✓	✓ ¹	×	×	×	✓	×
WRN ²	✓	✓	✓	✓	×	✓	✓

¹ Unique Zn²⁺-binding domain distinct from other RecQ conserved Zn²⁺-binding domains.

² Crystal structure of WRN helicase domain has not been solved, but indicated motifs are conserved.

Table 2

Chromosomal instability of human cells deficient in RecQ and Fe–S helicases

Family	Helicase	Associated disease/cancer	Chromosomal instability	References
RecQ	WRN	Werner syndrome	Chromosomal deletions and rearrangements; telomere loss	[64–66]
	BLM	Bloom syndrome	Elevated sister chromatid exchange	[67]
	RECQL1	Breast cancer	Elevated sister chromatid exchange	[68]
	RECQL4	Rothmund-Thomson syndrome, RAPADILINO, Baller–Gerold syndrome	Chromosomal rearrangements; aneuploidy	[69,70]
	RECQL5	Breast cancer	Chromosomal rearrangements; CPT-induced elevated sister chromatid exchange	[71,72]
Fe–S	FANCI	Fanconi anemia	Elevated sister chromatid exchange; MMC-induced chromosomal breakage; microsatellite instability	[73–76]
	RTEL1	Dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome	Telomere shortening and instability; anaphase bridges; trinucleotide repeat expansions	[77–82]
	DDX11	Warsaw Breakage syndrome	Sister chromatid cohesion defects; MMC-induced chromosomal breakage	[83,84]
	XPD	Xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy	UV-induced chromosome aberrations; abnormal chromosome segregation in XP-D and XP-D/CS cells	[85,86]

Abbreviations: CPT, camptothecin; MMC, mitomycin C; UV, ultraviolet light.

Table 3

Protein interactions of Fe–S cluster DNA helicases

Fe–S helicase	Protein partner	Interaction	References
DDX11	FEN-1	Stimulate 5' flap cleavage	[94]
	PCNA	ND	[94]
	Ctf18-RFC	ND	[94]
	Timeless (Tim)	Stimulate DDX11 helicase activity	[106]
	Ctf4	Recruit <i>Saccharomyces cerevisiae</i> Chl1 to replisome to facilitate cohesion	[105]
FANCD2	BRCA1	Mediate double-strand break repair by homologous recombination	[26]
	TopBP1	Facilitate DNA replication checkpoint upon replication stress	[108]
	RPA	Stimulate FANCD2 helicase activity	[38,109]
		Stimulate FANCD2 protein–DNA disruption activity	[110]
	MLH1	Promote cellular cross-link resistance	[111,112]
	BLM	Stimulate FANCD2 unwinding past DNA adduct	[113]
		Promote BLM protein stability	[113]
	FANCD2	Co-regulate interstrand cross-link-induced chromatin localization	[114]
		Promote FANCD2/FANCI stability	[115]
	RTT101	Facilitate DNA replication and promote telomere stability	[27]
RTT101	TRF2	Recruit RTT101 to telomere to promote T-loop unwinding	[116]
	CAK (MAT1)	Position CAK complex for transcription	[117]
XPB	CAK (MAT1)	Repress XPB helicase activity	[117]
		Stimulate XPB helicase activity	[93,118]
	p44	Stimulate <i>Chaetomium thermophilum</i> XPB ATPase/helicase activity	[21]

Abbreviations: ND, not determined.