

Chapter 6

DNA Helicases Associated with Genetic Instability, Cancer, and Aging

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Abstract DNA helicases have essential roles in the maintenance of genomic stability. They have achieved even greater prominence with the discovery that mutations in human helicase genes are responsible for a variety of genetic disorders and are associated with tumorigenesis. A number of missense mutations in human helicase genes are linked to chromosomal instability diseases characterized by age-related disease or associated with cancer, providing incentive for the characterization of molecular defects underlying aberrant cellular phenotypes. In this chapter, we discuss some examples of clinically relevant missense mutations in various human DNA helicases, particularly those of the Iron-Sulfur cluster and RecQ families. Clinically relevant mutations in the XPD helicase can lead to Xeroderma pigmentosum, Cockayne's syndrome, Trichothiodystrophy, or COFS syndrome. FANCD1 mutations are associated with Fanconi anemia or breast cancer. Mutations of the Fe-S helicase ChlR1 (DDX11) are linked to Warsaw Breakage syndrome. Mutations in the RecQ helicases BLM and WRN are linked to the cancer-prone disorder Bloom's syndrome and premature aging condition Werner syndrome, respectively. RECQL4 mutations can lead to Rothmund-Thomson syndrome, Baller-Gerold syndrome, or RAPADILINO. Mutations in the Twinkle mitochondrial helicase are responsible for several neuromuscular degenerative disorders. We will discuss some insights gained from biochemical and genetic studies of helicase variants, and highlight some hot areas of helicase research based on recent developments.

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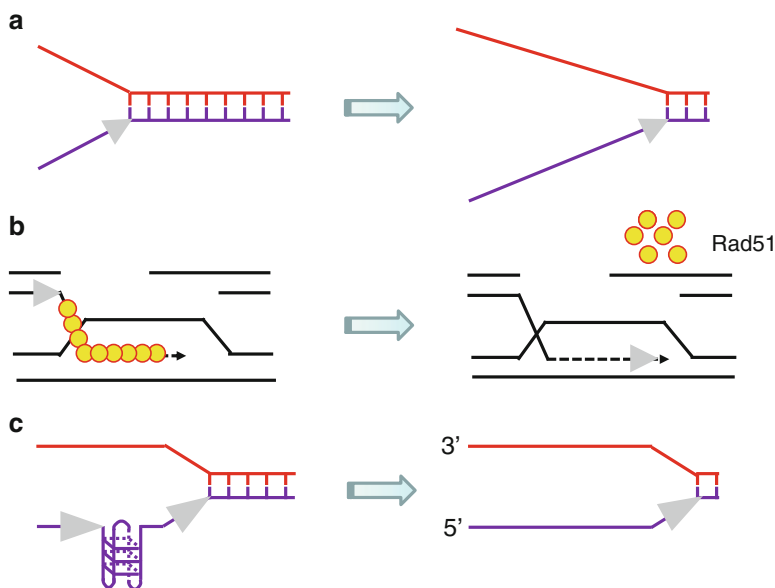


Fig. 6.1 Depending on their specialty, DNA helicases use the energy of ATP hydrolysis to perform multiple functions. (a) A DNA helicase can disrupt noncovalent hydrogen bonds between complementary strands of the DNA double helix to form transient single-stranded DNA tracts. (b) A DNA helicase can strip proteins (e.g., Rad51) off DNA to regulate HR. (c) A DNA helicase can resolve alternate DNA structures (e.g., G-quadruplex) to enable smooth progression of the replication fork

DNA Helicases: A Specialized Class of Molecular Motors

Helicases are molecular motors that couple nucleoside triphosphate (NTP) hydrolysis (typically ATP) to the unwinding of polynucleic acid structures [1, 2]. Helicases have multifaceted roles in virtually all aspects of nucleic acid metabolism, including replication, DNA repair, recombination, transcription, chromosome segregation, and telomere maintenance [2–6]. ATP-dependent DNA or RNA unwinding enzymes exist; however, certain helicases can act upon both DNA and RNA [6]. Although DNA helicases are conventionally known to unwind B-form DNA double helical molecules, some are specialized such that they can catalytically disrupt alternate DNA structures (e.g., D-loop, Holliday junction (HJ), triplex, G-quadruplex), strip proteins bound to either single-stranded or double-stranded DNA, perform chromatin remodeling, and/or anneal complementary single-strands [7–10] (Fig. 6.1).

Hereditary DNA Helicase Disorders

An increasing number of genetic diseases characterized by chromosomal instability are linked to mutations in DNA helicase genes. Many of these diseases are very rare, and inherited by autosomal homozygous recessive mutations. In certain cases,

mutations in human helicase genes are linked to premature aging or age-related diseases, whereas some helicase mutations have been associated with cancer. In addition to these, hereditary dominant mutations have been identified in the mitochondrial DNA helicase which lead to neuromuscular degenerative diseases. We will provide an overview on the clinical disorders associated with mutations in DNA helicase genes.

Iron-Sulfur Cluster DNA Helicase Diseases

A prominent family of DNA helicases with a linkage to human diseases that has acquired a great deal of interest is the so-called Iron-Sulfur (Fe-S) cluster helicases, named after a conserved metal binding domain in the helicase core [11, 12]. These helicases belong to the Superfamily 2 (SF2) grouping of DNA helicases which share sequence homology within the helicase core domain. Mutations in the Fe-S cluster helicase XPD, a key factor of the TFIIH complex implicated in transcription and nucleotide excision repair (NER), can give rise to multiple rare autosomal recessive clinical disorders: xeroderma pigmentosum (XP), XP combined with Cockayne's syndrome (CS), Trichothiodystrophy (TTD), and Cerebro-oculo-facial-skeletal (COFS) syndrome (for a recent review, see ref. [13]). Photosensitivity, neurological/developmental abnormalities, and skin cancer can be used to distinguish between XP, TTD, and CS; however, related or overlapping clinical features can arise from XPD mutations. XPD unwinds duplex DNA with a 5'-3' directionality, and together with the SF2 XPB helicase (which lacks an Fe-S cluster) is required for NER and transcription [14]. Operating as components of the TFIIH complex, two DNA helicases XPD and XPB with opposite translocation polarities are responsible for unwinding duplex DNA in the vicinity of the lesion to create a bubble containing the lesion, a prerequisite for proper removal of the damaged fragment [14]. In contrast to XPD where helicase activity is indispensable for NER, only XPB ATPase is necessary for the pathway to remove bulky lesions and UV photoproducts. XPB ATPase activity is also necessary for DNA opening for transcription to occur, but its helicase activity is important for promoter escape during transcription (for review, see ref. [15]).

Homozygous recessive mutations in the *FANCF* gene encoding the FANCF Fe-S cluster DNA helicase (also called BACH1 or BRIP1), and at least 14 other genes, are responsible for Fanconi anemia (FA), a disorder characterized by congenital defects, progressive bone marrow failure, cancer accompanied by chromosomal instability, and hypersensitivity to agents that induce DNA interstrand cross-links (ICLs) [16, 17]. Notably, *FANCF* mutations have also been associated with breast cancer [18]. Indeed, FANCF was originally discovered by its association with the tumor suppressor protein BRCA1 [19]. In addition to its role in ICL repair, FANCF helps cells to maintain genomic stability by resolving G-quadruplex (G4) DNA structures [20, 21], suggesting a more general role of the helicase in the response to replication stress.

The latest Fe-S cluster helicase implicated in a genetic disease is ChlR1, also known as DDX11, which is linked to Warsaw Breakage syndrome (WABS), a unique disease with cellular features of both FA and the cohesinopathy Roberts syndrome [22]. The clinical features of the single WABS patient reported include severe microcephaly, pre- and postnatal growth retardation, and abnormal skin pigmentation. Cells from the WABS patient display defective sister chromatid cohesion, a finding that is consistent with studies of mutant versions of ChlR1 homologs in yeast [23] and mouse [24] which also showed cohesion defects. The Lahti lab showed that depletion of human ChlR1 by RNA interference resulted in abnormal sister chromatid cohesion and a prometaphase delay leading to mitotic failure [25]. ChlR1 has a role in heterochromatin organization [26]; however, it is still unclear how the helicase is important for the cohesion process. Since cohesion is widely thought to be coupled to cellular DNA replication, it may be that ChlR1 is necessary for smooth replication fork progression through its catalytic activity and protein interactions, and that in its absence the cohesion proteins are not assembled properly; however, this may be an over simplistic assessment. Clearly further studies are necessary to understand the precise functions of ChlR1 required for chromosomal stability.

RecQ DNA Helicase Diseases

In addition to the Fe-S cluster DNA helicases, the SF2 RecQ DNA helicases are important for genomic stability and have been implicated in hereditary disorders. Homozygous recessive mutations in the *BLM* gene encoding a DNA helicase are responsible for Bloom's syndrome [27]. BS patients are highly sensitive to sunlight, immunodeficient, and display a broad spectrum of cancers early in life. The hallmark of BS is an elevated rate of sister chromatid exchange (SCE) [28]. BLM is believed to function in homologous recombination (HR) repair to maintain genomic stability. A model for the role of BLM in SCE suppression was proposed in which a BLM protein complex containing topoisomerase III α , RPA, RMI, and RMI2 dissolves double HJ structures which may form during recombination or the convergence of replication forks [29, 30]. BLM helicase activity working in concert with topoisomerase cleavage/ligation is required for the double HJ dissolution reaction. In addition to this function, BLM is believed to have other roles (early and late) in homologous recombination (HR) repair of double strand breaks (DSBs) [31, 32] and helping cells to deal with replication stress [33].

A second RecQ helicase disorder known as Werner syndrome (WS) is characterized by premature aging features and the early onset of age-related diseases such as cardiovascular disorders, diabetes mellitus (Type II), osteoporosis, and sarcoma and mesenchymal tumors. WS is characterized by genomic instability, sensitivity to DNA damaging agents, elevated recombination, and replication defects. The *WRN* gene encodes an RecQ 3'-5' DNA helicase and 3'-5' exonuclease that is proposed to play a role in regulation of recombination events, primarily HR [3, 34]. It may be

that WRN has a specialized function when the replication fork encounters a blocking lesion or alternate DNA structure. WRN interacts with a number of nuclear proteins, and these interactions are believed to facilitate genomic stability in various capacities including telomere maintenance, replication, and DNA repair [34, 35].

Mutations in a third RecQ family member, *RECQL4*, can lead to three distinct genetic disorders, namely Rothmund-Thomson syndrome (RTS), Baller-Gerold syndrome (BGS), and RAPADILINO (radial hypoplasia/aplasia, patellae hypoplasia/aplasia, cleft or highly arched palate, diarrhea, dislocated joints, little size (height at least 2 S.D. smaller than the average height) and limb malformation, nose slender, and normal intelligence) syndrome [36]. RTS patients are stunted in growth, and characterized by photosensitivity with poikiloderma, early graying and hair loss, juvenile cataracts, and osteogenic sarcomas. BGS is characterized by radial aplasia/hypoplasia and craniosynostosis, but not the poikiloderma typical of RTS patients [37]. Recent work from the Bohr lab has provided evidence that *RECQL4* has pleiotropic roles in cellular DNA metabolism that include DSB repair [38] and telomere maintenance [39]. *RECQL4* can be found in mitochondria where it helps to preserve the mitochondrial genome [40] and recruit p53 [41].

In addition to WRN, BLM, and *RECQL4*, two other human RecQ helicases, *RECQL1* (*RECQ1*) and *RECQL5* (*RECQ5*), are not yet linked to a disease; but it seems likely that these helicases will also play a role in cancer predisposition or a hereditary disorder characterized by chromosomal instability [42]. Indeed, studies of primary fibroblasts from *RECQ1*-knockout mice [43] and human cells depleted of *RECQ1* by RNA interference [44] show chromosomal instability. Recent evidence has implicated distinct roles of *RECQ1* (and *RECQL4*) in DNA replication initiation [45]. *RECQ5* was found to be associated with RNA polymerase and may help to maintain genomic stability during transcription [46, 47]. *RECQ5* also participates in DNA decatenation through its cooperation with Topoisomerase II alpha [48].

Twinkle Mitochondrial DNA Helicase

Aside from the Fe-S and RecQ DNA helicases, mutation of the Twinkle mitochondrial DNA helicase co-segregates with a number of diseases with mitochondrial defects including adult-onset progressive external ophthalmoplegia, hepatocerebral syndrome with mtDNA depletion syndrome, and infantile-onset spinocerebellar ataxia [49]. Twinkle is required for replication of human mitochondrial DNA, and mutations in the *C10orf2* gene encoding Twinkle helicase can lead to mitochondrial deletions in post-mitotic tissues. Work from several laboratories demonstrated that Twinkle is an oligomeric helicase that unwinds double-stranded DNA molecules [50–52], and can also catalyze strand annealing [51], a function that is also observed for a number of the RecQ helicases [42]. It is yet unclear what the biological significance of helicase-catalyzed strand annealing truly is, but may play a role when replication forks are stalled or during DSB repair.

Hereditary Missense Mutations in DNA Helicase Disorders

Structural and biochemical analyses of purified recombinant DNA helicase proteins has sparked tremendous interest in understanding the molecular pathology behind disease-causing mutations in this specialized class of molecular motor ATPases. Disease-causing missense mutations result in single amino acid substitutions have been identified in DNA helicase disorders, which may be insightful for understanding biochemical mechanism and cellular function. In the following section, we will discuss some basic lessons from the spectrum of clinically relevant missense mutations in human DNA helicase genes, highlighting some unique aspects and potential areas of investigation.

Disease-Causing Missense Mutations in Iron-Sulfur Cluster DNA Helicases

XPD Missense Mutations

Missense mutations in the *XPD* gene are linked to four hereditary diseases: XP, XP combined with CS, TTD, and COFS syndrome with some cases of partially overlapping clinical features [13] (Fig. 6.2). *XPD* mutations responsible for XP are located mainly in the helicase core domain, and either significantly reduce or completely inactivate helicase function. The XP-causing *XPD* mutations do not affect basal transcription [53], consistent with a requirement of *XPD* ATPase/helicase activity for NER, but not transcription. As components of the TFIIH complex, *XPD* helicase operating in conjunction with the opposite polarity *XPB* helicase unwinds double-stranded DNA in the vicinity of the helix distorting lesion to create a bubble that can be acted upon by structure-specific nucleases to remove a short (~30 nt) single-stranded fragment containing the damage. Repair synthesis and ligation complete the steps of NER.

XPD mutations responsible for XP/CS also reside within or near the conserved helicase motifs as well (Fig. 6.2). However, *XPD* missense mutations causing XP/CS are believed to interfere with *XPD* protein interactions within the TFIIH complex. Unlike the *XPD* mutations implicated in XP or XP/CS which are only found in or very near the helicase core domain, *XPD* mutations responsible for TTD can also be found in the C-terminal region of the protein (Fig. 6.2). The C-terminus of *XPD* is important for interaction with other proteins (e.g., p44 subunit of TFIIH), which can be critical for optimal *XPD* helicase activity and/or stability of the TFIIH complex (for review, see ref. [54]). Such mutations in *XPD* reduce DNA repair activity and basal transcription [55]. Several *XPD* missense mutations linked to TTD that have been examined inhibit basal transcription, suggesting a molecular defect distinct from that of XP.

The heterozygous *XPD* mutations identified in a COFS syndrome patient were a *R616W* null mutation previously observed in an XP patient and a unique *D681N*

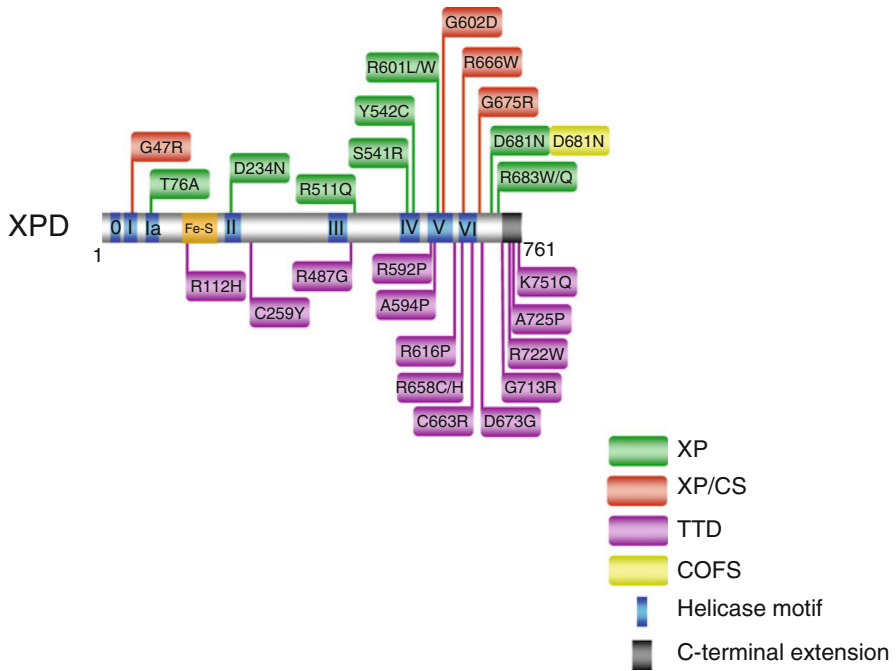


Fig. 6.2 Clinically relevant missense mutations in XPD helicase responsible for Xeroderma pigmentosum, Xeroderma pigmentosum combined with Cockayne syndrome, Trichothiodystrophy, and COFS Syndrome. See text for details. For discussion of *XPD* mutations and genetic heterogeneity, see ref. [13]. *XPD-D681N* mutation is associated with XP and COFS syndrome

mutation residing on the C-terminal side of motif VI [56] (Fig. 6.2). The aspartic acid (D681) is highly conserved in all known *XPD* genes. UV survival assays performed with COFS syndrome patient cells showed UV sensitivity comparable to that of cells from an XP-A patient with severe XP. It is unclear how the *XPD-D681N* mutation can result in distinct clinical phenotypes of COFS syndrome vs. XP. The wide spectrum of *XPD* missense mutations resulting in heterogeneity in clinical phenotype prompt continued interest to characterize the molecular and cellular defects of *XPD* variants to acquire a better understanding of the relevant diseases.

FANCI Missense Mutations

The great majority of *FANCI* missense mutations genetically linked to FA or associated with breast cancer reside in the N-terminal portion of the protein where the helicase core domain is located (Fig. 6.3), suggesting that ATP-dependent DNA unwinding by *FANCI* is required for its function in the FA pathway of DNA repair and its involvement as a tumor suppressor. The requirement of *FANCI* helicase

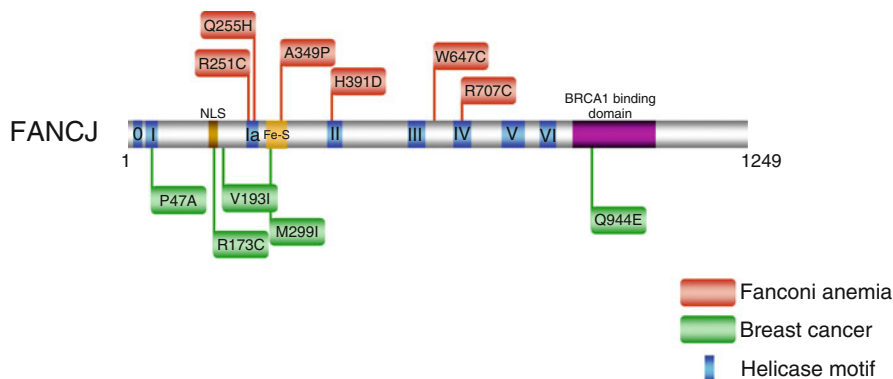


Fig. 6.3 Clinically relevant missense mutations in FANCD1 helicase responsible for Fanconi Anemia complementation group J and associated with breast cancer. See text for details. For a comprehensive listing of *FANCD1* mutations, see The Rockefeller University-Fanconi anemia mutation database www.rockefeller.edu/fanconi/mutate; also see ref. [18]

activity for proper operation of the FA pathway is consistent with genetic complementation studies in human cells which show that cross-link resistance is dependent on catalytically active FANCD1 protein [57].

A FANCD1 missense mutation linked to FA of particular interest is an alanine-to-proline substitution in the Fe-S domain at residue 349 immediately adjacent to a highly conserved cysteine that is important for binding an iron atom [58] (Fig. 6.4). Inheritance of the paternal *FANCD1-A349P* missense allele and a maternal truncating *R798X* allele resulted in phenotypic abnormalities, including intrauterine growth failure and death as a stillborn fetus with a gestational age of 22 weeks [58]. Biochemical analysis of purified recombinant FANCD1-A349P protein demonstrated that it was defective in coupling ATP-dependent DNA translocase activity to unwinding duplex DNA or displacing proteins bound to DNA [59] (Fig. 6.4). From a genetic standpoint, expression of the *FANCD1-A349P* mutant allele failed to rescue sensitivity of FANCD1 null cells to a DNA cross-linking agent or the G-quadruplex (G4) binding drug telomestatin, indicating that it was unable to perform its role in ICL repair or resolution of G4 DNA [59]. In addition, expression of the *FANCD1-A349P* allele in a wild-type background exerted a dominant negative effect on resistance to DNA cross-linkers or telomestatin. Thus, the ability of FANCD1 to couple ATP hydrolysis and DNA translocase activity to higher order functions such as unwinding structured nucleic acids or stripping protein from DNA is essential for its biological roles.

There is one FANCD1 missense mutation, Q944E, identified in a breast cancer patient that is positioned outside the helicase core domain (Fig. 6.3). The Q944E mutation resides in the BRCA1 binding domain located in the C-terminal region of FANCD1 (Fig. 6.2). Given the relatively close linear proximity of residue Q944 to S990, the site for FANCD1 phosphorylation required for the physical interaction between FANCD1 and BRCA1 [60], it will be of interest to determine if the BRCA1

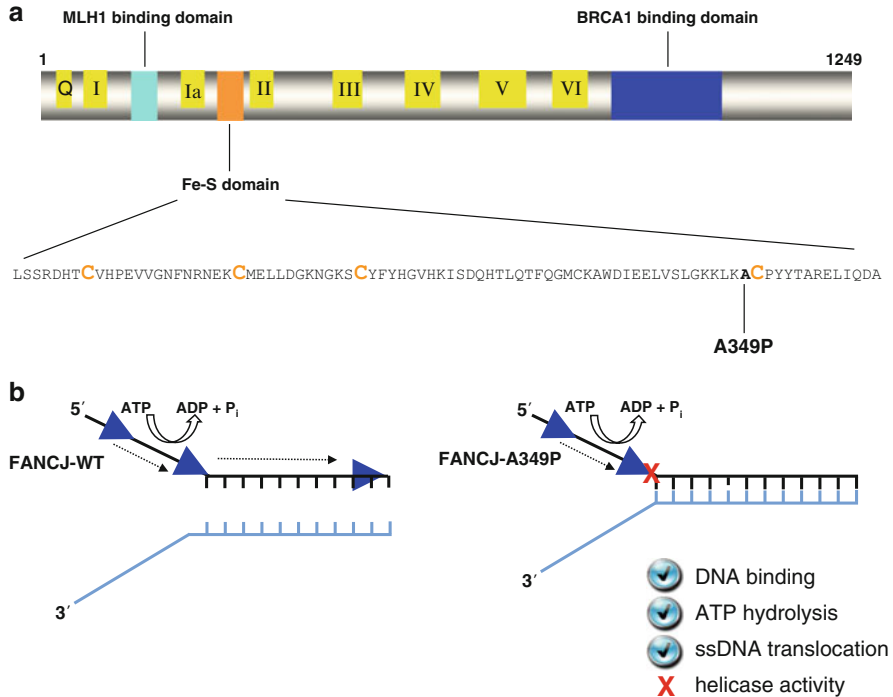


Fig. 6.4 An FA complementation group J patient mutation (A349P) in the conserved Fe-S domain uncouples DNA ATPase and translocase activities from strand separation (helicase) activity. **(a)** FANCD1 protein with the conserved helicase core domain, key protein interaction domains, and the Fe-S cluster. The conserved helicase motifs are indicated by *yellow boxes*, and the protein interaction domains for MLH1 and BRCA1 are shown by *aqua green* and *blue boxes*, respectively. The expanded Fe-S domain shows the locations for conserved cysteine residues in *orange*, and the A349P missense mutation of a FANCD1 patient in *bold*. **(b)** The purified recombinant FANCD1-A349P protein fails to couple ATPase and single-stranded DNA translocase activity to unwinding duplex DNA. See text and ref. [59] for details

binding to FANCD1 is affected by the Q944E mutation. Such a FANCD1 missense mutation may influence the molecular mechanism employed for DNA repair, as it was recently shown that disruption of the FANCD1-BRCA1 interaction blocks DNA repair by homologous recombination and promotes $\text{pol}\eta$ -dependent bypass [61].

DDX11 (ChlR1) Single Amino Acid Deletion Mutation

Mutations in the ChlR1 helicase (also named DDX11) are genetically linked to the chromosomal instability disorder WABS [22]. The WABS patient is characterized by a compound heterozygous *K897del* mutation in trans with a maternally inherited splice site mutation in an intron that leads to a truncated protein, leading to

nonsense-mediated decay of the maternal allele and monoallelic expression of the *K897del*-containing paternal allele. The *K897del* mutation results in a single amino acid deletion of a lysine residue that resides ten amino acids from the C-terminus of the protein. The endogenous hChlR1-K897del protein was poorly detected by immunoblot analysis of lysates from fibroblasts or lymphoblasts of the affected individual [22]. Cells from the patient exhibit chromosomal instability characterized by sister cohesion defects, chromosomal breakage, and sensitivity to the DNA cross-linking agent mitomycin and topoisomerase inhibitor camptothecin. Based on the cellular phenotypes, it was suggested that WABS is a unique disease with cellular features of both FA and the cohesinopathy Roberts syndrome.

Biochemical analysis of the purified recombinant ChlR1 protein demonstrated that it possesses a DNA-dependent ATPase activity and a 5′–3′ helicase activity [62, 63]. The purified recombinant hChlR1-K897del protein was dramatically inhibited for DNA binding and DNA-dependent ATPase activity compared to the wild-type recombinant ChlR1 protein [64], suggesting an important role of the extreme C-terminus of the protein for its stable interaction with nucleic acid. Further cellular and biochemical studies are likely to yield insight to the proposed role of the ChlR1 helicase to allow smooth replication fork progression necessary for proper sister chromatid cohesion during mitosis [62]. The strong preference of ChlR1 to unwind G4 DNA may be important for stability at particular genomic loci [64]. Greater insight to the disease will likely be gained from more biochemical and genetic studies, including the identification and characterization of other mutant *ChlR1* alleles.

Disease-Causing Missense Mutations in RecQ DNA Helicases

BLM Missense Mutations

Inspection of the clinical spectrum of Bloom's syndrome patient missense mutations reveals that the ones identified to date reside in the helicase core domain or the adjacent RecQ C-terminal region (RQC) [65] (Fig. 6.5). This mutational pattern strongly suggests that BLM helicase activity is required for suppression of the BS disease phenotype, a result that is consistent with the observation that expression of an engineered Walker A box (motif I) catalytically inactive BLM protein failed to complement the genomic instability of BS cells; moreover, the helicase-dead BLM protein exerted a dominant negative effect on cell viability in a wild-type background [66]. In fact, all of the BS patient-derived helicase domain mutant proteins that have been biochemically characterized show defects in ATP or DNA binding, which result in low or no detectable helicase activity [66–69].

The conserved RQC region in BLM, found in many RecQ helicases, consists of a Zn²⁺ binding domain and a winged helix domain. Two of the cysteine residues (C1036 and C1055) residing in the RQC region and responsible for binding Zn²⁺ are mutated in BS (Fig. 6.5). Five different amino acids substitutions (S, G, R, V, and Y)

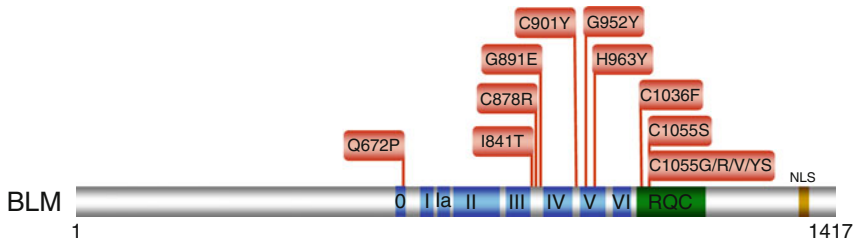


Fig. 6.5 Clinically relevant missense mutations in BLM helicase responsible for Bloom’s syndrome. See text for details. For a comprehensive listing of *BLM* mutations, see The Bloom’s Syndrome Registry www.med.cornell.edu/bsr/; also see ref. [65]

resulting from BLM patient mutations occur at C1055, suggesting that this particular locus in the BLM Zn^{2+} domain may be a hotspot for mutation and that the cysteine residue is highly important for BLM structure and function. Biochemical characterization of the purified recombinant BLM-C1055S mutant protein showed that it lacked ATPase and helicase activity [66, 67]. Expression of the BLM-C1055S mutant protein *in vivo* failed to rescue the p53-mediated apoptosis defect of BS fibroblasts [70]. Collectively, the location of the BLM missense mutations and their molecular analysis demonstrate that BLM helicase activity is required for its *in vivo* function to suppress BS phenotypes.

RECQL4 Missense Mutations

The RECQL4 helicase is distinct from other human RecQ helicases because three distinct genetic disorders can be attributed to mutations in the *RECQL4* gene: RTS, RAPADILINO, and BGS [36]. Unlike BLM, missense mutations for all three RECQL4 diseases have been identified in regions outside the conserved helicase domain shared by the other RecQ helicases [36] (Fig. 6.6). RTS missense mutations are found in the C-terminal region, N-terminal region, and the helicase core domain. Only two RAPADILINO missense mutations have been reported, one in the N-terminal region and one in the helicase core domain. For BGS, a single patient missense mutation (R1021W) is known that resides in the C-terminal region. Interestingly, the C-terminal R1021W mutation was genetically linked to both BGS and RTS. The effects of the R1021W mutation, as well as all of the other mutations in the *RECQL4* gene, on the biochemical functions of the RECQL4 helicase protein have not yet been determined.

WRN Missense Mutations

The discovery that mutations in the *WRN* gene are linked to a premature aging disorder [71] sparked a tremendous amount of interest in understanding the

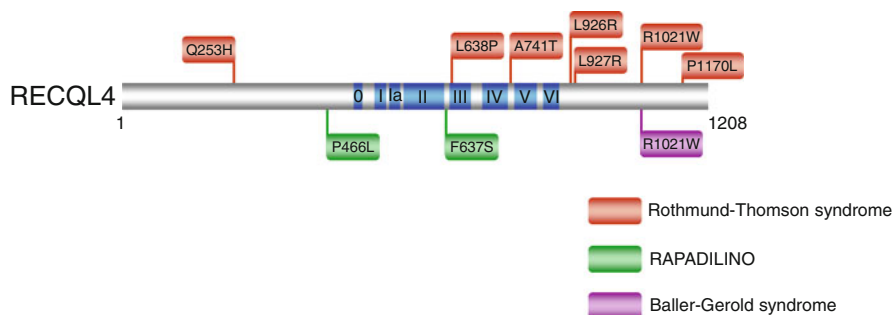


Fig. 6.6 Clinically relevant missense mutations in *RECQL4* helicase responsible for Rothmund-Thomson syndrome, Baller-Gerold syndrome, and RAPADILINO. See text for details. For comprehensive listing of *RECQL4* mutations, see ref. [36]. *RECQL4*-R1021W is associated with both RTS and BGS

pathological basis for the disease on a molecular level; however, the clinical spectrum of WS mutations was rather uninformative because those identified resulted in truncated proteins. Since the nuclear localization sequence (NLS) of WRN protein residing in the extreme C-terminus, it would be predicted that these WRN protein fragments would fail to localize to the nucleus where WRN is required to preserve genomic stability [72]. However, more recently a small number of *WRN* missense mutations have been identified (Fig. 6.6). One of these, the *M1350R* missense mutation would be predicted to interfere with the ability of the WRN protein to effectively localize to nuclei since it resides immediately N-terminal to the NLS of WRN [73].

Two missense mutations reside in the N-terminal exonuclease domain of WRN were shown to result in WRN protein instability [74]. From a research standpoint, this is unfortunate because if the mutant WRN proteins had been selectively inactivated for exonuclease function, they may have been useful for understanding the molecular importance of that catalytic function. Two additional WRN missense mutations were found in the helicase domain, one in the Walker A box (motif I) (*G574R*) [73] just three amino acids away from the invariant lysine residue implicated in nucleotide binding and the other (*R637W*) [75] very near helicase motif V. Although not yet biochemically characterized, these two helicase core domain mutants would be predicted to interfere with normal ATPase and helicase function. If this were true, they may constitute separation of function mutations that would be valuable for assessing the relative importance of WRN helicase vs. exonuclease activity to suppress the cellular phenotypes associated with WS. Genetic and biochemical studies suggest the dual importance of balanced and concerted WRN helicase and exonuclease activities [76–78]; however, there is still much to learn precisely how WRN helicase or exonuclease activities suppress the disease phenotypes of WS.

Disease-Causing Missense Mutations in the XPB and Twinkle DNA Helicases

XPB Missense Mutations

The *XPB* gene encodes a DNA helicase with opposite polarity to that of XPD that is also found in the TFIIH complex, and *XPB* mutations can lead to multiple clinical disorders including XP/CS, XP with neurological abnormalities, and TTD [13]. In contrast to XPD where helicase activity is indispensable for NER, only XPB ATPase is necessary for the pathway to remove bulky lesions and UV photoproducts. XPB ATPase activity is also necessary for DNA opening for transcription to occur, but its helicase activity is important for promoter escape during transcription (for review, see ref. [15]). It is generally believed that the phenotypic heterogeneity of XPB is attributed to missense mutations which only partially affect XPB biochemical and cellular function in mild XP/CS, whereas severe XP/CS is associated with nonsense mutations in both *XPB* alleles, resulting in altered and reduced XPB protein [79]. A causative mutation in the XPB helicase resulting in a single amino acid substitution (T119P) in the N-terminus of the protein prior to the conserved helicase motifs was identified in a patient with mild versions of both TTD and photosensitivity [80]. Cellular studies from the Sarasin lab demonstrated that the *XPB-T119P* allele is associated with moderately defective DNA repair [81]. This is in contrast to *XPB-F99S* allele (also located in the N-terminus) of an XP/CS patient which is profoundly reduced in its DNA repair function [81]. Interestingly, both the T119P and F99S mutations in XPB do not impair TFIIH helicase activity [15]; however, the F99S substitution was shown to impair the interaction of XPB with p52, one of the subunits of the TFIIH complex [55]. Collectively, the studies of TFIIH and clinical spectrum of mutations in the XPB and XPD helicases have helped to dissect the complex heterogeneity of DNA repair-transcription syndromes.

Twinkle Missense Mutations

As mentioned earlier, missense mutations in the human *C10orf2* mitochondrial helicase gene encoding Twinkle DNA helicase are linked to several neuromuscular degenerative disorders. Expression of patient-derived Twinkle mutant proteins in normal cells or transgenic mice led to the accumulation of mitochondrial DNA replication intermediates and mitochondrial DNA depletion [82–84], consistent with the idea that dysfunctional Twinkle helicase can cause mitochondrial replication forks to stall. The reduction in Twinkle helicase activity for those variants that were examined correlated to the extent of mitochondrial DNA depletion and accumulation of replication intermediates [82]. Biochemical analysis of 20 disease variants of the human mitochondrial DNA helicase showed that the mutations are quite

heterogeneous in terms of their molecular effects on ATPase and helicase function as well as effects in some cases on protein stability [85]. All 20 mutant Twinkle variants retained at least partial helicase activity, leading the authors to propose that these defects are consistent with the delayed presentation of mitochondrial diseases associated with the *c10orf2* mutations.

Recent Advances and Research Directions for DNA Helicases Implicated in Disease

The helicase field is moving at a brisk pace, with the development of new technologies and a greater understanding of their biological roles. In the following section, we will highlight some interesting developments from our viewpoint. These emerging stories, as well as others discussed in the accompanying chapters, convey a very exciting era for the scientific community beyond the scope of DNA metabolism because there are rippling implications for the fields of aging, disease, and cancer.

Communication Between Redox Active DNA Repair Proteins

Nearly all DNA repair pathways share a few common steps: (1) initial detection of a DNA lesion; (2) processing of the damaged DNA nucleotide/strand; (3) removal of the damaged nucleotide/strand; (4) replacement of the damaged nucleotide/strand with correct nucleotide/sequence; (5) sealing the nick(s) by ligation. Of these steps, initial detection of the lesion is critical and can be rate-limiting due to low copy number of DNA damage recognition proteins and lack of preferential DNA binding affinity for the lesion compared to undamaged sequence. Recent experimental evidence supports a model that DNA charge transport is a signaling mechanism for the recruitment of redox active DNA repair proteins to the vicinity of DNA damage ([86, 87], and for review see ref. [88]). In the latest development, Sontz et al. [89] provide experimental evidence that coordinated DNA charge transport between DNA repair proteins with redox active Fe-S clusters, the XPD NER helicase and EndoIII base excision DNA glycosylase, can occur to enable efficient redistribution of the repair proteins to the location of DNA damage within a vast excess of undamaged nucleotides. Thus, DNA repair proteins (or proteins involved in other aspects of DNA metabolism such as replication or gene expression) conventionally thought to operate in distinct pathways might collaborate with each other in an unexpected way by their ability to participate in DNA charge transport chemistry. Although there is evidence for cross-talk between DNA repair pathways and also with replication

[90–93], the ability of redox active proteins to collaborate by DNA charge transport broadens the scope of possibilities since the proteins do not necessarily need to physically interact with one another. This is highly provocative for the potential synergy that might exist between redox active DNA interacting proteins that influence diverse pathways previously thought to function independently of one another. Research that further explores the collaboration between proteins able to perform DNA charge transport will likely have important implications for molecular gerontology and cancer biology.

Coordination of End-Processing Proteins in Double Strand Break Repair Resection

DSBs can be highly poisonous and lethal to cells; therefore, several DNA repair pathways including nonhomologous end-joining and homologous recombination exist to join ends back together again. In replicating cells, the preferred mechanism of DSB repair is by homologous recombination because it occurs with high fidelity, using the sister chromatid duplex as a template. Based on experimental evidence, BLM is implicated in an early stage of DSB repair in which strand resection from a DNA end occurs to provide a 3' single-stranded DNA tail for RAD51-mediated strand invasion into recipient duplex [32, 94, 95]. Strand resection is much more complex than previously thought, and requires a battalion of helicases, nucleases, DNA binding proteins, and additional accessory factors. The BLM helicase promotes access of the DNA end to either DNA2 or EXO-1, enabling processive catalytic removal of one strand to produce the 3' single-stranded DNA tail.

Another layer of complexity for the involvement of BLM in strand resection was suggested by the discovery that BLM interacts with FANCD1 [96], which is associated with BRCA1, a tumor suppressor molecule implicated in DSB repair [19]. FANCD1-deficient cells have a defect in DSB-induced HR and show delayed resolution of DSBs following ionizing radiation [97]. Moreover, FANCD1 and certain other HR proteins function downstream of FANCD2/I mono-ubiquitination in ICL repair to presumably repair the processed DNA cross-link by an HR-mediated pathway. We propose a model in which strand resection is facilitated by FANCD1 and BLM helicases translocating on opposite strands of the broken double-stranded DNA end to promote access to structure-specific nucleases (EXO-1, DNA2) [98]. The FANCD1-BLM partnership may facilitate resection at chemically modified blocked DNA ends or through G-rich sequences prone to form G-quadruplex (G4) structures. This model for the dual collaboration of FANCD1 and BLM in DSB repair can be tested by a combination of genetic and biochemical approaches. DNA end resection assays in a reconstituted system with chromatinized templates may help to elucidate the functional importance of the FANCD1-BLM interaction in DSB repair in a biological context.

Structural Determinants of XPD Helicase Function and Translocation Polarity

Recent progress in understanding the molecular architecture of Fe-S cluster DNA helicases has come from several labs working on archaeal XPD proteins. This effort began with the discovery that XPD bears an Fe-S cluster metal binding site essential for its DNA unwinding activity, but not necessary for binding single-stranded DNA or ATP hydrolysis [99]. Crystal structures solved independently by three research groups confirmed the existence of a novel Fe-S domain [100–102]. The XPD structure contains two Rad51/RecA-like domains (HD1 and HD2) with two additional domains, the Fe-S and Arch domains, inserted between adjacent β -strands of the central β -sheet of HD1. ATP binding and hydrolysis controls the conformational state of the Fe-S and Arch domains in conjunction with the conserved helicase motifs that comprise the helicase core domain. The Fe-S domain was proposed to form a wedge with the nearby Arch domain to separate the DNA duplex as the enzyme translocates in an ATP-dependent manner. Mutations introduced to the Fe-S domain, including the conserved cysteines, abolished XPD helicase activity and/or destabilized tertiary structure [99, 100], attesting to the structural importance of the Fe-S domain. Biochemical studies demonstrated that the integrity of the XPD Fe-S domain is required for the proper folding and structural stability, and is important for coupling ATP hydrolysis to unidirectional translocation [103]. Furthermore, the Fe-S cluster serves to stabilize elements of protein secondary structure and target the helicase to the single-stranded/double-stranded DNA junction [103].

The structure of XPD in complex with a short DNA fragment was recently reported [104]. This provided a working model for the mechanism of translocation by Fe-S cluster DNA helicases. The XPD-DNA crystal structure, combined with a mutational and biochemical analysis of XPD, revealed how the 5'–3' directionality of translocation along DNA is achieved and suggested how the XPD enzyme might act upon a DNA substrate harboring a helix-distorting lesion susceptible to NER. These conclusions on how regulation of translocation polarity by XPD helicase is achieved were further supported by another very recent study using proteolytic DNA and mutational analysis of XPD [105]. Based on these two studies, it was suggested that Fe-S domain helicases achieve a polarity of ATP-driven translocation opposite to that of 3'–5' helicases by conformational changes within the motor domain rather than binding single-stranded DNA with an opposite orientation. It is plausible that the Fe-S redox activity may provide an additional mechanism for FANCDJ and other DNA repair molecules to communicate with each other and sense DNA-mediated charge transport such that they cooperate with each other to assemble and/or translocate with a defined polarity and in a regulated manner [106]; however, this hypothesis remains to be experimentally tested.

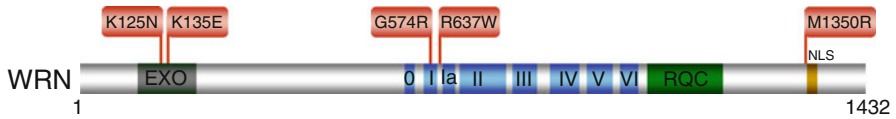


Fig. 6.7 Clinically relevant missense mutations in WRN helicase-nuclease responsible for Werner syndrome. See text for details. For a comprehensive listing of *WRN* mutations, see The International Registry of Werner Syndrome www.wernersyndrome.org; also see ref. [73]

Small Molecule Inhibitors of DNA Helicases

Understanding the precise molecular-genetic defects of helicase disorders in some cases such as the RecQ diseases can be challenging. Partial functional redundancy between DNA helicases as well as backup pathways make it difficult to establish cause and effect relationships between a helicase defect and biological outcome. Since DNA helicases provide essential functions in multiple steps of DNA damage response and repair pathways, it will be highly informative to precisely characterize their functions *in vivo*. This information will lead to new insight to their roles in prevention or correction of genomic DNA damage that is a causative force for cellular senescence and contributes to organismal decline associated with aging. Use of small molecules to target human helicases for inhibition (or activation) in a cell-based model system is a novel approach to the problem of assigning specific helicase functions *in vivo*. In addition to the new insight to the roles of helicases in DNA damage response and repair to prevent age-associated phenotypes, this work will also be informative for the development of anticancer strategies which target DNA repair proteins/processes [107].

Recently, a small molecule (NSC 19630) from the NCI Diversity Set was identified that inhibited WRN helicase activity, but not other DNA helicases tested, suggesting specificity [108]. Human cells exposed to NSC 19630 were dramatically impaired in their growth and proliferation, and displayed elevated apoptosis in a WRN-dependent manner, suggesting an effect of the small molecule that mimicked expression of a helicase-dead protein. Furthermore, cellular exposure to the WRN helicase inhibitor resulted in elevated γ -H2AX and proliferating cell nuclear antigen (PCNA) foci, and delayed S-phase progression, consistent with the accumulation of stalled replication forks. Exposure to NSC 19630 sensitized cancer cells to agents that induce replication stress or interfere with DNA repair. We anticipate that the WRN helicase inhibitor molecule may be helpful in understanding WRN-mediated pathway(s) important for the response to DNA damage and replication stress (Fig. 6.7).

Summary

In this chapter, we have attempted to provide an overview of hereditary helicase disorders, with a particular emphasis on clinically relevant missense mutations as these may prove to be valuable in understanding pathway dysfunction. In addition,

we have highlighted some hot topics in helicase research that we believe will be important for future studies. Clearly, progress in understanding the biochemical and genetic functions of RecQ and Fe-S cluster helicases will serve as a springboard for new investigations. In addition, the recent progress in understanding the molecular basis for Twinkle mitochondrial helicase disorders has created a lot of excitement in the field. Contributions from an increasing number of scientists will continue to unveil new insights to the roles of DNA helicases in suppressing genomic instability, cancer, and age-related diseases.

Acknowledgments This research was supported by the Intramural Research Program of the NIH, National Institute on Aging and the Fanconi Anemia Research Fund (RMB). We apologize to helicase researchers whose work was not cited due to space limitations.

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