Cell. Mol. Life Sci. 66 (2009) 1209 – 1222
1420-682X/09/071209-14
DOI 10.1007/s00018-008-8580-6

Cellular and Molecular Life Sciences

Review

Welcome the Family of FANCJ-like Helicases to the Block of Genome Stability Maintenance Proteins

Y. Wu, A. N. Suhasini and R. M. Brosh, Jr.*

Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, NIH Biomedical Research Center, 251 Bayview Blvd, Baltimore, MD 21224 (USA), Fax: 410-558-8157, e-mail: broshr@mail.nih.gov

Received 17 September 2008; received after revision 24 October 2008; accepted 28 October 2008
Online First 19 December 2008

Abstract. The FANCJ family of DNA helicases is emerging as an important group of proteins for the prevention of human disease, cancer, and chromosomal instability. FANCJ was identified by its association with breast cancer, and is implicated in Fanconi Anemia. Proteins with sequence similarity to FANCJ are important for maintenance of genomic stability. Mutations in genes encoding proteins related to FANCJ, designated ChlR1 in human and Chl1p in yeast, result in sister chromatid cohesion defects. Nematodes mutated in *dog-1* show germline as well as somatic deletions in genes containing guanine-rich DNA. *Rtel* knockout mice are embryonic lethal, and embryonic stem cells show telomere loss and chromosomal instability. FANCJ also shares sequence similarity with human XPD and yeast RAD3 helicases required for nucleotide excision repair. The recently solved structure of XPD has provided new insight to the helicase core and accessory domains of sequence-related Superfamily 2 helicases. The functions and roles of members of the FANCJ-like helicase family will be discussed.

Keywords. Fanconi anemia, DNA repair, helicase, genomic stability, G quadruplex, FANCJ, XPD, CHL1, CHLR1, DOG1, RTEL.

Introduction

The identification of *FANCJ* mutations in early-onset breast cancer patients [1] and Fanconi anemia (FA) group J patients [2–4] implicates FANCJ as a tumor suppressor caretaker that ensures genomic stability. Emerging evidence indicates that FANCJ is important for DNA interstrand cross-link (ICL) repair [4], the response to replicational stress [5], and resolution of alternate G-quadruplex DNA structures [6]. Interestingly, DNA helicases or putative helicases that share sequence similarity with FANCJ from a number of organisms have important roles in the DNA damage response, DNA repair, and the maintenance of genomic stability. We will summarize the collective findings of FANCJ-like helicases, highlighting some of the most recent discoveries of this intriguing class of DNA unwinding enzymes.

The N-terminal 888 amino acids of FANCJ contain the ATPase/helicase core domain, which shares sequence similarity with a number of putative or bona fide DNA helicases belonging to a DEAH subfamily of Superfamily 2 (SF2) helicases [1]. A number of these proteins have been shown to be important for genomic stability in a variety of organisms (Fig. 1A). Interestingly, representatives from each of these organisms play important roles in DNA repair, chromosome

* Corresponding author.

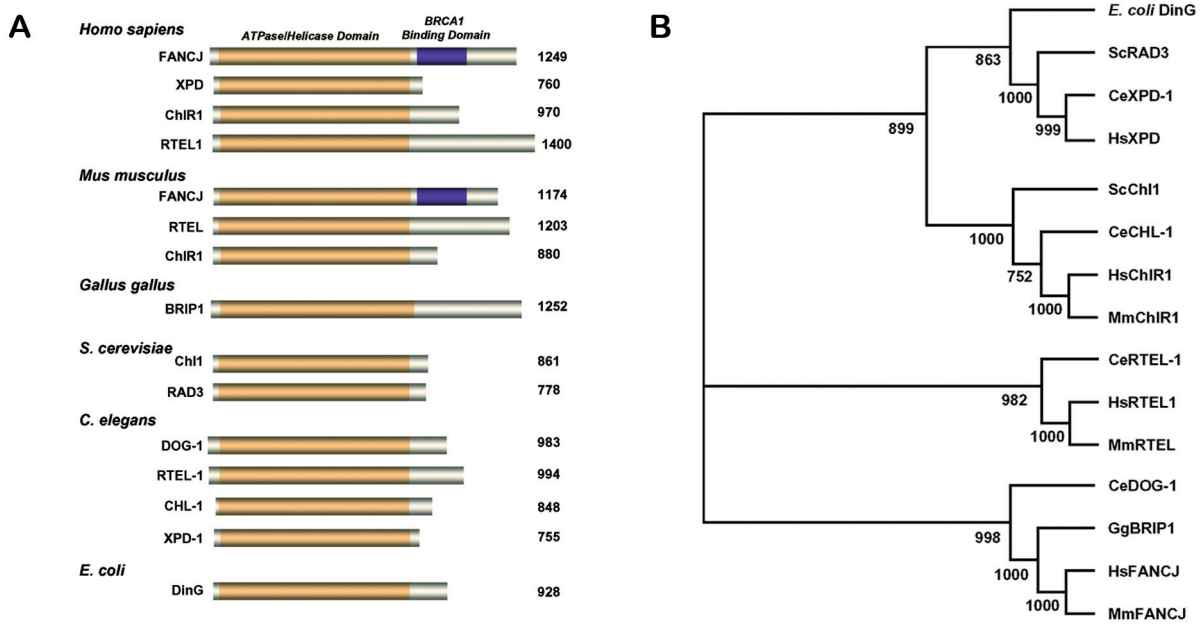

Figure 1. Alignment and phylogenetic tree of FANCJ-like proteins across species. *Panel A*, The conserved helicase core domain is indicated by yellow. The BRCA1 binding domain for FANCJ is indicated with purple. The number of amino acids for each helicase is indicated on the right. *Panel B*, The amino acid sequences of the full length proteins in (*A*) were aligned and a phylogenetic tree was constructed by ClustalX2 with number of bootstrap trials at 1000. The image was generated in Treeview. Branch numbers refer to bootstrap values.

segregation, and/or genome integrity. However, as discussed below, only human and mouse FANCJ proteins contain a BRCA1 interaction domain located in the C-terminus that is important for its DNA repair function (Fig. 1A). A phylogenetic analysis of these FANCJ-like helicases reveals that mammalian FANCJ is most closely related by sequence to chicken BRIP1 and worm DOG-1 (Fig. 1B), consistent with findings that these proteins are FANCJ orthologs [7, 8]. Proteins more distantly related to FANCJ by sequence similarity are represented by the XPD, ChlR1 and RTEL groups (Fig. 1B).

In this review, we will discuss our current understanding of the significance and function of FANCJ-like helicases in cellular DNA metabolism, with an emphasis on their structural features, biochemical and genetic functions, and importance in human disease. We will begin with a brief description of FANCJ's clinical importance, and the molecular-genetic pathways that it is believed to operate in. We will then proceed to describe the biological importance of DNA helicases or putative helicases sharing sequence similarity with FANCJ, and what is known about their cellular or molecular functions.

FANCJ Helicase, Breast Cancer, and the Fanconi Anemia Pathway

FA is an autosomal recessive disorder characterized by multiple congenital anomalies, progressive bone marrow failure, and high cancer risk [9–11]. Cells from FA patients exhibit spontaneous chromosomal instability and hypersensitivity to DNA ICL agents. Although the precise mechanistic details of the FA pathway of ICL repair are not well understood, progress has been made in the identification of the FA proteins that are required for the pathway. For a detailed description of the FA pathway and the FA proteins, the reader is referred to several recent reviews [9–11]. Among the 13 FA complementation groups from which all FA genes have been cloned, only a few of the FA proteins are predicted to have direct roles in DNA metabolism. One of the more recently identified FA proteins shown to be responsible for complementation of the FA complementation group J is FANCJ [2–4]. FANCJ was originally designated BACH1 (BRCA1 Associated C-terminal Helicase), which was discovered by Cantor et al. as a protein that binds to the BRCT repeats of BRCA1 [1]. A genetic interaction between FANCJ and BRCA1 in double-strand break (DSB) repair was established [1], and *FANCJ* mutations were identified in early onset breast cancer [1, 12, 13], suggesting a tumor suppressor role of FANCJ.

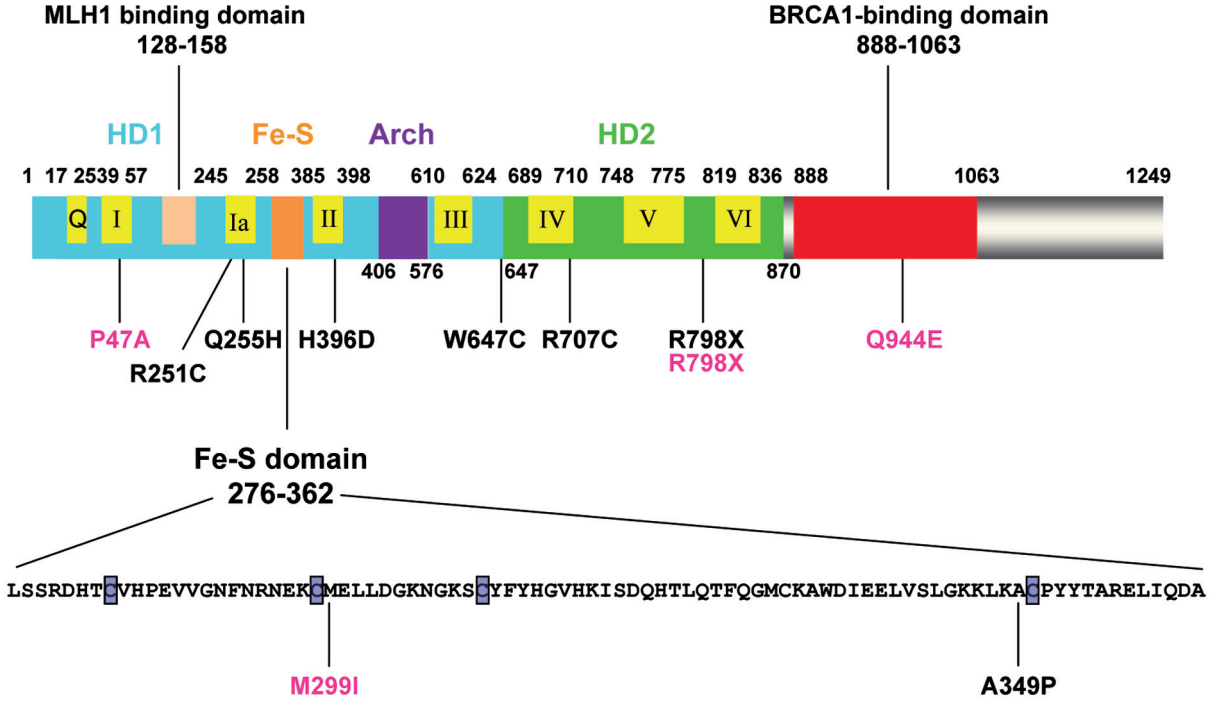

Figure 2. Conserved helicase core, accessory, and protein interaction domains of FANCJ, and the distribution of *FANCJ* mutations and breast cancer associated sequence changes. The conserved helicase motifs are indicated by yellow boxes. The purposed crystal structure HD1 domain is shown in cyan, Fe-S domain in orange, Arch domain in purple, and HD2 domain in green. MLH1 binding domain is indicated in tan, and BRCA1 interaction domain in red. The Fe-S domain is expanded and the four conserved cysteines are indicated with blue. FA-causing mutations are marked in black below the protein schematic, and breast cancer associated mutations are marked in pink.

FANCJ coding sequence mutations genetically linked to FA (Complementation Group J) [2, 3] or associated with breast cancer [1, 12, 13] are shown in Fig. 2. The LOVD FA Database (http://www.rockefeller.edu/fanconi/mutate/) contains an updated list of FANCJ coding alterations, including naturally occurring polymorphic variants of unknown clinical importance. All *FANCJ* mutations genetically linked to FA are found within the helicase core domain, which includes the conserved ATPase/helicase motifs and the regions between the motifs as well as the conserved Iron-Sulfur (Fe-S) cluster domain found in a number of the FANCJ-like helicases. A truncating *FANCJ* mutation (R798X) within the helicase core domain that causes FA in biallelic carriers confers susceptibility to breast cancer in monoallelic carriers [13] (Fig. 2). FANCJ missense mutations associated with breast cancer were identified in motif I, the Fe-S domain, and the BRCA1 interacting domain (Fig. 2). Interestingly, *FANCJ* missense mutations genetically linked to FA or associated with breast cancer reside immediately adjacent to the second and fourth cysteine residues in the conserved Fe-S domain, respectively (Fig. 2).

Molecular Functions and Protein Partners of FANCJ

FANCJ was first shown by Cantor and colleagues to be a DNA-dependent ATPase that catalytically unwinds duplex DNA with a 5' to 3' directionality [14]. Consistent with its directionality, FANCJ requires nucleic acid continuity in the 5' ssDNA tail of the forked duplex substrate near the ssDNA-dsDNA junction to efficiently initiate DNA unwinding [15]. Further analysis demonstrated that FANCJ preferentially binds and unwinds forked duplex substrates, but requires a 5' ssDNA tail [15], unlike certain RecQ helicases which can unwind synthetic replication fork substrates lacking any preexisting ssDNA in the structure [16]. Although FANCJ fails to unwind a Holliday Junction structure, the helicase unwinds D-loop structures by releasing the third strand of the homologous recombination (HR) intermediate [15]. The activity of FANCJ on a D-loop substrate may be relevant to its proposed role in a HR pathway of DSB repair [1, 4, 9]. *FANCJ* null cells have an HR defect [4], and FANCJ-depleted cells are mildly sensitive to ionizing radiation (IR) and have delayed resolution of IR-induced DSBs [17]. The sensitivity of *FANCJ* null cells to DNA ICL agents [4] may be a result of a defective DNA metabolic event of the FA pathway

that occurs downstream of FANCD2 monoubiquitination [4, 7]. BRCA1 is required for the transport of FANCJ, BARD1, BRCA2, and Rad51 to sites of DNA damage where other proteins such as the MRE11-RAD50-NBS1 complex associate [18]. The assembly of a BRCA1/FANCJ/BARD1 complex enables the interaction of BRCA1/FANCJ with TopBP1, a factor that plays an important role in the execution of the S phase checkpoint [18]. The ability of RAD51 foci to form in FA-J cells [4] suggests that FANCJ does not operate upstream of Rad51 foci formation during HR repair. Rather, FANCJ may disrupt Rad51 nucleoprotein filaments in order to complete HR repair or prevent untimely or promiscuous recombination between sequences that are not perfectly homologous. FANCJ exists in a protein complex with mismatch repair proteins MLH1-PMS2 (MutLα) [19]. FANCJ directly interacts with MLH1 independent of BRCA1, and this DNA-independent interaction was mapped to a defined 30 amino acid region of the FANCJ helicase domain (Fig. 2). Genetic studies demonstrated that FANCJ helicase activity and MLH1 binding, but not BRCA1 binding, were essential to correct the sensitivity of FA-J cells to agents that induce ICL and consequently interfere with cell cycle progression, resulting in G2/M accumulation [19]. Although mismatch repair proteins have been implicated in the sensing and/or processing of ICLs, this study provided the first evidence for a direct link between FA and mismatch repair, leading to the prediction that FANCJ has a broader role in DNA damage signaling independent of BRCA1 [19].

In addition to its direct involvement in DNA repair, some evidence suggests that FANCJ may also have a specialized role under conditions of replicational stress. FANCJ shows increased association with chromatin during S phase [5], and co-localizes with RPA in response to DNA damage or replicational stress [20]. RPA physically and functionally interacts with FANCJ, stimulating its DNA unwinding activity [20]. Activation of FANCJ helicase activity is required for timely progression through S phase of the cell cycle [5]. Potential blocks to the replication fork include DNA damage, static protein-DNA complexes, and alternate DNA structures. A helicase-like protein with sequence similarity to the FANCJ helicase domain in nematodes was discovered to have a role in guanine-rich-DNA metabolism [21] (discussed in detail below), raising the possibility that FANCJ has a similar function to resolve secondary structures *in vivo*. Guanine-rich nucleic acids, which are abundant in the human genome [22], have the potential to form G-quadruplex (G4) DNA stabilized by Hoogsteen hydrogen bonding between guanine residues [23]. G-quadruplex structures may impede cellular DNA replication or transcription, as well as play a role in immunoglobulin gene rearrangement, promoter activation, and telomere maintenance [23]. Biochemical studies with the purified FANCJ protein demonstrated that the helicase unwinds a variety of G-quadruplex DNA structures in an ATP hydrolysis dependent manner [6]. FANCJ-depleted cells treated with the G4-interactive compound telomestatin displayed impaired proliferation and elevated levels of apoptosis and DNA damage compared to control cells, suggesting a model in which G4 DNA is a physiological substrate acted upon by FANCJ to facilitate DNA replication (Fig. 3) [6]. Further studies that establish defined relationships between cellular defects and *FANCJ* mutations will lead to a greater understanding of the pathological basis for FA and the roles of FANCJ in FA-dependent as well as FA-independent pathways.

Biological Studies of FANCJ orthologs

In this section, we will summarize some key findings from genetic studies of FANCJ orthologs in chicken and nematode. Although mouse *FANCJ* was deposited in GenBank (Accession No. Q5SXJ3), no biological study has yet been reported.

Chicken Ortholog of FANCJ. To examine the genetic functions of FANCJ in a model system, the Hiom lab generated a *FANCJ* (*brip1*) knockout in DT40 cells [7]. The chicken ortholog of FANCJ contains 55% identical residues and 70% overall similarity with human FANCJ, the greatest similarity being in the conserved helicase core domain with 68% identity and 80% similarity. However, the chicken FANCJ sequence does not contain the C-terminal BRCA1 interaction domain found in human FANCJ (Fig. 1A), and the percent identity and similarity outside the helicase core domain are 25% and 44%, respectively. *brip1* mutant DT40 cells were found to be highly sensitive to the DNA-crosslinking agent cisplatin, and expression of human FANCJ could rescue the *brip1* sensitivity [7]. Intact ATPase, but not BRCA1 interaction domain, was required for human FANCJ rescue of the chicken *brip1* mutant cells. *brip1* mutant cells showed intact FANCD2 ubiquitination, indicating that chicken FANCJ has a function in the FA pathway that operates downstream of FANCD2 activation. *brip1* mutant cells were not sensitive to IR and were proficient in HR of a DSB induced by the SceI restriction endonuclease in a reporter gene. A major conclusion from these studies using the chicken DT40 cells was that FANCJ has a function in the FA pathway that is independent of BRCA1. This con-

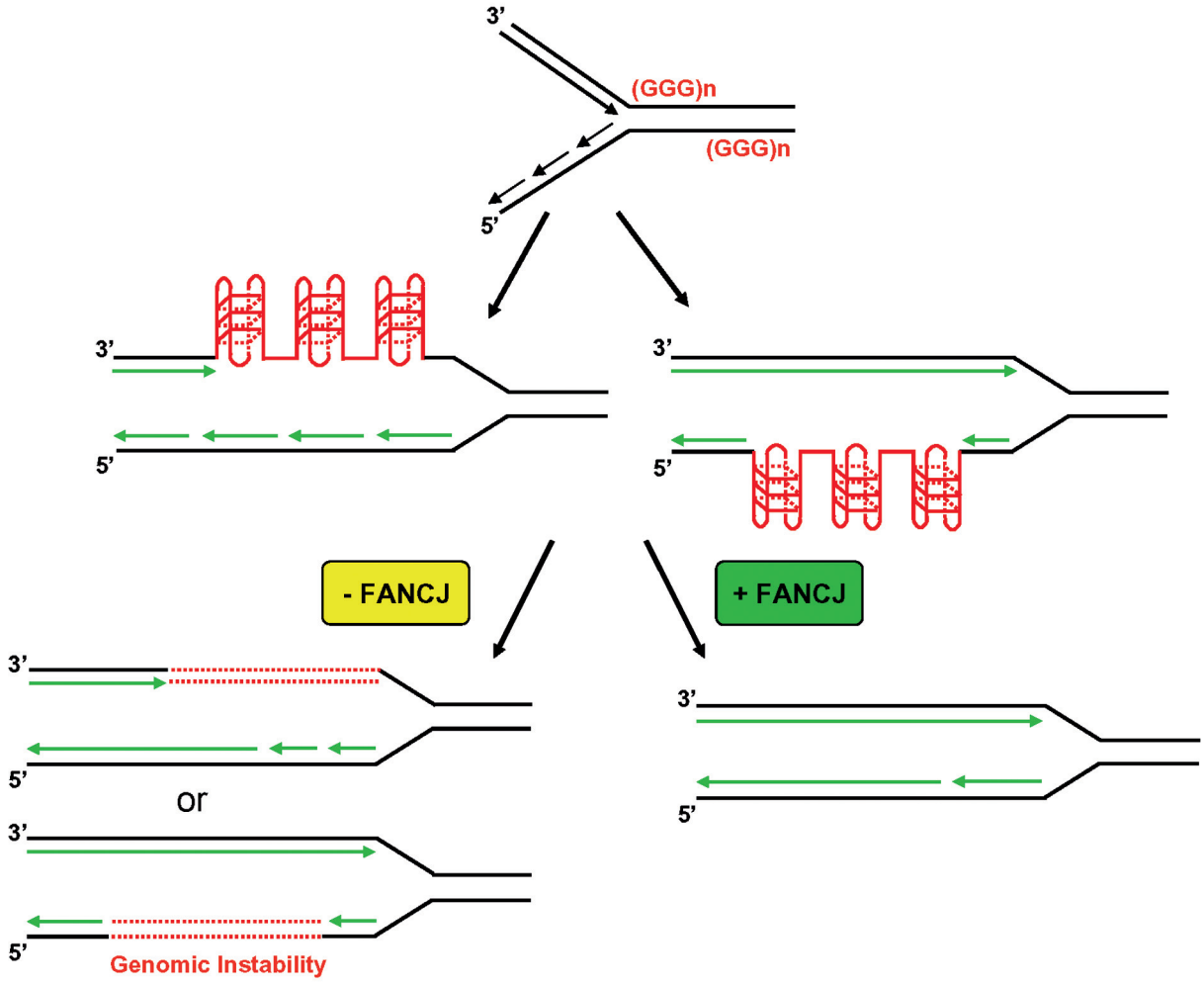

Figure 3. Proposed role of FANCJ in resolving G-quaduplex DNA during replication. According to the model presented, the replication fork moves in from the left toward the poly(G) sequences (in red). DNA synthesis using either the leading-strand or lagging-strand template is postulated to occasionally give rise to stable secondary structures such as G-quadruplexes. FANCJ helicase is recruited to unwind the G4 structure, permitting polymerization to continue to synthesize nascent DNA (in green). Failure to resolve G-quadruplex DNA in FA-J cells results in genomic instability (dashed line in red).

clusion is supported by the results of Peng et al. which show that FANCJ binding to BRCA1 is not important for resistance of human cells to ICL agents [19].

DOG-1 Helicase-like Protein Preserves G-tract Stability and Functions in ICL Repair in C. elegans

The *dog-1 (deletions of guanine-rich DNA)* gene in *C. elegans* encodes a protein sharing 32% identity and 51% similarity with FANCJ in the conserved helicase core domain; however, DOG-1 protein lacks the BRCA1 interaction domain (Fig. 1) and no significant sequence similarity with FANCJ outside the helicase core domain exists [21]. The Lansdorp lab discovered that mutations in the *C. elegans dog-1* gene result in deletions of several hundred nucleotides that initiate near the 3' end of the G tract in polyG/polyC tracts (> 18 nt) throughout the worm's genome [21]. These deletions extend upstream of the G tract for various lengths, but are not observed at consecutive A/T tracts. Acute depletion of DOG-1 by RNA interference exerted a similar effect on genomic stability as that observed in *dog-1* mutants [21]. The peculiar type of genomic instability in *dog-1* animals led the authors to propose that DOG-1 might be a helicase, capable of resolving secondary structures that form in G-rich sequences of the nematode's genome. However, no biochemical activities of the DOG-1 protein have been reported, and the *dog-1* gene product is yet to be shown to have helicase activity that would act on secondary structure in G/C tracts.

To assay the *in vivo* contribution of various DNA repair genes to G/C tract stability of *dog-1* mutants, Youds et al. examined the genomic instability of DNA repair mutants in a *dog-1* background [24]. Mutation

of the *BLM* ortholog *him-6* in the *dog-1* background elevated the G/C tract deletion formation, whereas mutation of the *WRN* homolog *wrn-1* in the *dog-1* background had no effect. HR repair genes (*BARD1, RAD51, XPF*) were found to contribute to the prevention of deletions in *dog-1* mutants. Using an RNA interference approach in *dog-1* mutants, it was observed that reduction of trans-lesion synthesis polymerases polη and polκ stimulated deletion induction at monoG tracts as well [24]. Characterization of G/C tract instability in *dog-1* mutants that also contained a mutation in a variety of DNA repair genes led the authors to suggest that inactivation of components of nonhomologous end joining (NHEJ), HR, or single-strand annealing (SSA) did not suppress deletion formation in a *dog-1* background [24]. Thus the elevated level of mutation events in the *dog-1* mutant is not formed through these repair pathways.

Kruisselbrink *et al.* [25] developed a reporter-based assay to measure the deletions at monoG tracts in *dog-1* mutants and found that deletions occurred in *dog-1* deficient animals; however, neither the wild-type animals nor mono A tracts were susceptible for deletions. Further characterization of the G-tract instability of *dog-1* mutants revealed that the deletion inducing capacity of G-rich DNA increased with tract length and G-ratio. Deletions were detected in the range of 63–7347 nucleotides; however, the size was less than 300 bp in a large percentage of the deletions. Sequence homology at the deletion junctions was not observed, suggesting that a mechanism of microhomology-dependent DSB repair at G4 DNA-blocked replication forks does not occur in the *dog-1* mutant.

Until very recently, characterization of the genomic instability in *dog-1* mutants was primarily based on PCR assays that were directed with specific primers that would detect deletions initiating within the G/C tracts. To expand the range of mutations analyzed, a combination of techniques including genetic mapping, SNP mapping, and oaCGH (oligo array Comparative Genome Hybridization) were used to analyze the *dog-1* mutant, leading Zhao and colleagues to identify large chromosomal rearrangements including duplications, deletions and translocations in addition to small deletions previously observed [26].

The Rose lab demonstrated that *C. elegans* DOG-1 is the functional homolog of FANCJ [8]. This conclusion was based on their observations that *dog-1* mutants were highly sensitive to ICL agents and exhibit chromosomal abnormalities after DNA cross-linking. Genetic analysis showed that *dog-1* is epistatic with *fcd-2* (*C. elegans* FANCD2), but not epistatic with *brc-1* (*C. elegans* BRCA1), suggesting that DOG-1 operates in the same pathway as FANCD2. In contrast, DOG-1 and BRCA1 function in different pathways for the repair of ICLs. DOG-1 is dispensable for FANCD2 focus formation at sites of replication stress, suggesting that DOG1 operates downstream of FANCD2, similar to what is observed in human cells for FANCJ [4, 7]. DOG-1 is also dispensable for RAD51 focus formation [8], an observation that was also made for FANCJ in human cells [4]. The broader spectrum of mutational events observed as a consequence of *dog-1* mutation suggests that similar chromosomal instabilities that might occur in FANCJ-deficient human cells would be a critical determinant of cancer susceptibility in FA patients. Biochemical analysis of DOG-1 protein and determination of its protein partners and DNA targets will provide a better understanding of its cellular functions.

Other SF2 Helicases with Sequence Similarity to the FANCJ Helicase Core Domain

In this section, we will describe genetic, biochemical, and structural aspects of other DNA helicases or putative helicases that share sequence similarity with FANCJ in the helicase core domain, but are not considered as functional homologues of FANCJ. The determination of the first 5' to 3' SF2 helicase structure provides new insights to the biochemical functions of the XPD and related helicases. Genetic and cellular studies in yeast, mouse, and human cells demonstrate the biological importance of other helicases related to FANCJ by the conserved helicase core domain.

Chl1p Helicase Plays a Critical Role in Sister Chromatid Cohesion and Genomic Integrity

Several studies showed that the yeast DNA helicase-like protein Chl1p prevented chromosome loss or missegregation [27–31], but the mechanism was not well understood. The activated checkpoint of *chl1* mutants, characterized by a G2-M phase delay, suggests that the protein has a role before the completion of mitosis [27]. *chl1p* mutations which abolish ATP binding were observed to inactivate its function in chromosome segregation [32]. In 2004, the Skibbens lab reported that Chl1p, which shares sequence similarity in the core helicase domain with FANCJ and related helicases, is required for proper sister chromatid cohesion [33]. During sister chromatid cohesion, newly replicated sister chromosomes are physically linked together by a protein complex known as cohesin to permit their appropriate distri-

bution to daughter cells in anaphase [34]. A genetic interaction between *chl1* and two genes (*CTF7/ECO1*, *CTF18/CHL12*) that function in sister chromatid cohesion was demonstrated, and Chl1p was found to physically interact with the cohesion factor Ctf7p/Eco1p, further supporting a role for Chl1p in the fidelity of chromosome segregation [33]. In addition to Chl1p, budding yeast require a specialized replication factor C (*Ctf18/Dcc1/Ctf8*) and DNA polymerase associated Ctf4 to maintain sister chromatid cohesion in cells arrested for long periods during mitosis [35]. *Chl1* was identified as a high copy suppressor of *ctf18Δ* spore lethality, and CHL1 was found to be essential for chromatid disjunction during meiosis II, consistent with its role in sister chromatid cohesion [35]. More recently, it was shown that *chl1Δ* is synthetically lethal with *ctf18Δ* [36]. Dosage increase of *chl1+* rescued the sensitivity of *swiΔ*, a mutant of the "replication fork protection complex", to agents that induce replicational stress, pointing toward a role of Chl1p in the S phase stress response and maintenance of replication forks. Interactions of yeast Chl1p with components of the replication machinery [35, 37], together with the genetic evidence, implicate a role of Chl1p to preserve genomic stability by promoting proper chromosome segregation and efficient sister chromatid cohesion during S phase, presumably related to its ability to stabilize replication forks.

The first evidence that a role of Chl1p in mammalian cells might be conserved was garnered by the Lahti lab that identified two highly related human genes, *DDX11* and *DDX12* encoding the proteins hChlR1 and hChlR2, respectively, which share sequence similarity with yeast Chl1p [38, 39]. hChlR1 and hChlR2 were found to be only expressed in proliferating human cell lines; moreover, quiescent human fibroblasts that reenter S phase display concomitant expression of the hChlR1 and hChlR2 proteins with PCNA [39]. Interestingly, *DDX11* was also found to be a strong candidate gene for telomere length determination in humans [40]. Studies with human cells showed that hChlR1 dynamically relocalizes from diffuse mitotic chromatin in prophase to concentrated regions at spindle poles during the metaphase transition [41]. hChlR1 depletion resulted in abnormal sister chromatid cohesion and a pro-metaphase delay leading to mitotic failure [41]. Consistent with its role in sister chromatid cohesion, hChlR1 was found to reside in a complex with cohesion factors [41]. Subsequent RNA interference studies with human cells showed that hChlR proteins are required for proper chromosome cohesion at both the centromeres and along the chromosome arms, and tight binding of cohesion complexes to chromatin [42]. The Androphy lab went on to examine the role of mitotic chromosome associated ChlR1 in the maintenance of replicating papillomavirus viral episomes [43]. They determined that ChlR1 loads papillomavirus E2 protein onto mitotic chromosomes in a kinetochore-independent mechanism for viral genome attachment to mitotic chromosomes and ultimately, maintenance of the viral genome. It was proposed that ChlR1 may serve as a target for eradication of the virus during human infection.

To examine the potential importance of Chl1 during development and mitosis, a *DDX11* mutant mouse was made [42]. Loss of ChlR1 in mouse resulted in embryonic lethality. The aneuploidy apparent in *DDX11-/-* embryos is a consequence of sister chromatid cohesion defects and placental malformation. Partial loss of sister chromatid cohesion in *chlr1*-deficient cells leading to accumulation of aneuploid cells in *chlr1*-null mouse embryos suggests a mechanism that would contribute to tumorigenesis.

The Lahti lab provided the first biochemical characterization of a ChlR1 protein member, demonstrating that recombinant hChlR1 purified from insect cells is a DNA-dependent ATPase with a preferred cofactor of ssDNA [44]. hChlR1 efficiently unwound a partial duplex DNA substrate of 19 bp as well as a DNA:RNA hybrid, but the enzyme is not very processive based on its poor ability to unwind a DNA duplex of 41 bp. Based on results using helicase DNA directionality substrates, the authors suggested that hChlR1 preferentially translocates 5' to 3' on short ssDNA templates, whereas hChlR1 can translocate in both directions when the partial duplex substrate has a long intervening ssDNA region; however, the 5' to 3' direction is clearly preferred [44].

More recently, the Hurwitz lab purified a recombinant FLAG-tagged hChlR1 that was expressed in human 293 cells and characterized the protein's biochemical activities [45]. hChlR1 displayed DNA-dependent ATPase activity and a 5' to 3' helicase directionality. hChlR1 unwound partial duplexes of 100 bp, and in the presence of RPA or the Ctf18-RFC complex could unwind duplexes of 500 bp. hChlR1 was shown to interact with Ctf18-RFC, PCNA, and FEN-1, and hChlR1 stimulated FEN-1 endonuclease activity on an equilibrating flap structure, a model substrate intermediate that forms during lagging strand synthesis [45]. Depletion of hChlR1 or FEN-1 resulted in defective sister chromatid cohesion [45]. The genetic and biochemical interactions of yeast Chl1p or human ChlR1 and other cohesion establishment factors with gene products implicated in lagging strand processing suggests that the two processes are related, or perhaps coupled to one another. In a model proposed by the Hurwitz lab, the concerted action of cohesion factors

and replication proteins at the fork allows completely processed and ligated Okazaki fragments in a sister chromatid complex to be passed through the cohesion ring, insuring that the two sister chromatids remain attached until mitosis. Thus, ChlR1's involvement in lagging strand processing may be important for sister chromatid cohesion.

There is mounting evidence that sister chromatid cohesion defects lead to tumorigenesis. Furthermore, a link between the tumor suppressor BRCA1 and sister chromatid pairing reactions has been suggested, placing an emphasis on this field of research since the prognosis for cure of BRCA1-dependent basal-type breast cancer patients, which accounts for 45% of hereditary breast cancer, is generally poor [46].

The involvement of BRCA1-interacting helicase FANCJ and the related helicase hChlR1 in DNA replication or repair intimately associated with the fidelity of sister chromatid cohesion has engaged an interest in the roles and pathways of these DNA unwinding enzymes. It will be important to understand how defects in BRCA1 and FANCJ promote chromosome abnormalities characteristic of cancer cell phenotypes in adult tissues at the molecular level. In addition to somatic cell cancer, mutations in cohesion establishment factors can also lead to diseases characterized by developmental abnormalities (e.g., Cornelia de Lange Syndrome, Roberts Syndrome) [46], suggesting a role of helicase-dependent processes important for proper sister chromatid cohesion during embryogenesis, an idea that is supported by the *chlr1*-null mouse studies [42].

Role of RTEL in Telomere Metabolism

Interestingly, certain proteins sharing sequence similarity with FANCJ in their helicase core domain have been proposed to also play a role in telomere metabolism. In addition to human *DDX11* encoding the hChlR1 protein discussed above, the *Rtel* (*R*egulator of *te*lomere *l*ength) gene product has been implicated in the maintenance of telomere length or capping based on the observation that embryonic stem cells from *rtel* knockout mice show telomere loss and display many chromosome breaks and fusions upon differentiation [47]. The *rtel* knockout mice die between 10 and 11.5 days of gestation with defects in the nervous system, heart, vasculature, and extraembryonic tissues [47]. To examine the importance of RTEL in postnatal development, Wu *et al.* [48] generated conditional knockout alleles for the *rtel* gene through Cre-mediated recombination and crossed these mice with a ubiquitous Cre transgenic line. The progeny of this cross displayed embryonic defects identical to those observed for *Rtel* null embryos. It has been proposed that the G-rich 3' tail of telomeres may be a target for the helicase to resolve alternate G-quadruplex structures that might form, enabling the telomere structure to be properly replicated or repaired. However, very little is known about the molecular mechanism for RTEL functions and its predicted role in G-tract or telomere metabolism.

Using genetic and biochemical approaches, Barber *et al.* demonstrated that *C. elegans* RTEL-1 is a functional analog of yeast Srs2 [49]. Like *srs2*, *rtel-1* mutant worms display hyperrecombination, DNA damage sensitivity, and synthetic lethality with the *sgs1* homolog BLM (*C. elegans him-6*) which correlates with an accumulation of recombination intermediates. Depletion of RTEL in human cells by RNA interference resulted in decreased HR and sensitivity to mitomycin C, which introduces ICLs, but not increased IR sensitivity. The ability of human RTEL to unwind D-loop recombination intermediates but not disrupt Rad51-ssDNA protein filaments suggests that RTEL acts as an anti-recombinase in a specialized manner to insure genomic stability [49].

Lessons from Archaea XPD Structure: Functional Helicase Domains and Molecular Defects in Human Diseases

The FANCJ-like family of DNA helicases includes the human XPD and yeast RAD3 helicases [1], which are subunits of the TFIIH transcription/DNA repair complex required for nucleotide excision repair (NER) and transcription initiation [50, 51]. Mutations of human XPD cause three diseases: xeroderma pigmentosum (XP), trichothiodystrophy (TTD), and combined XP with Cockayne's syndrome (XP/CS) [52], indicating the clinical significance of XPD functions and mutations. XP patients display prominent sun sensitivity, and elevated ($>$ 1000-fold) frequency of sun-induced skin cancer. Both CS and TTD are multisystem disorders with a wide range in type and severity of symptoms. CS patients display mainly neurological and developmental abnormalities, and TTD patients typically show brittle hair and scaly skin, and some neurological and developmental impairments reminiscent of CS [53]. Cells from XP donors are hypersensitive to UV irradiation, which is mainly caused by defects of NER [54]. Conversely, TTD cells show more transcriptional deficiencies [55]. A number of studies have characterized the catalytic ATPase and DNA helicase functions of XPD, and XPD protein interactions [56]. XPD, a core component of the transcription and repair factor TFIIH, is an 87-kDa ATP-dependent helicase that unwinds DNA

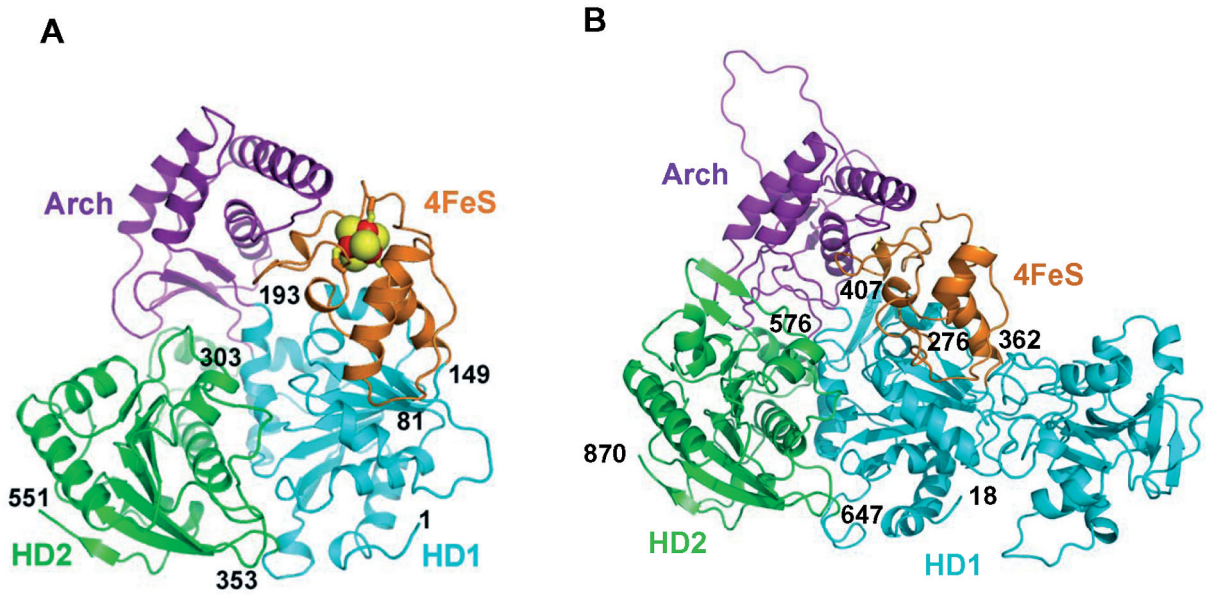

Figure 4. Structure of SaXPD (*Panel A*) and homology model of human FANCJ based on SaXPD (*Panel B*). *Panel A*, Front view shows SaXPD helicase domains HD1 (cyan) and HD2 (green) which form the ATP-binding interface. The Fe-S (orange) and Arch (purple) domains form the Arch shape. The Fe metal ions (red) bound by cysteines (yellow) in Fe-S domain are shown. XPD structure provided by Drs. L. Fan and J. Tainer [61]. *Panel B*, Homology model of human FANCJ was generated by superimposing FANCJ amino acids 18–870 corresponding to conserved helicase core domain onto SaXPD structure. Sequence alignment was made by ClustalX2, FANCJ protein model was generated using Swiss-Model, and image was produced using PyMol.

in the 5'–3' direction [57]. Point mutations that knock out the ATPase or helicase activities of XPD have a limited effect on transcription initiation by TFIIH but abolish NER, suggesting its helicase activity is essential for NER, but not transcription initiation [58]. XPD protein-protein interactions are critical for both helicase activity and stability of the TFIIH complex [56]. TTD-causing mutations in the XPD carboxyl terminus that weakens XPD binding to TFIIH subunit p44 impairs damaged DNA opening activity of XPD [59]. The TFIIH complex from a TTD patient has basal transcription defects *in vitro* as well as reduced *in vivo* TFIIH concentration [60], suggesting a role of XPD in TFIIH stability that is impaired by the TTD-causing mutation.

In an effort to relate the genetic causes of *XPD* mutations with molecular defects, and to better understand the functions of XPD, several groups set out to solve the crystal structure of XPD. Due to the difficulty of purifying human XPD in the absence of its interacting factors, XPD homologs from Archaea were exploited for structural studies. Very recently, three groups solved the XPD crystal structure from archaea *Sulfolobus acidocaldarius* (SaXPD) (Fig. 4A) [61], *Sulfolobus tokodaii* (StXPD) [62], and *Thermoplasma acidophilum* (TaXPD) [63]. In the following discussion, we will summarize some major features of the XPD domains, highlighting the two RecA-like domains, the Fe-S cluster, and the Arch domain. For reference, please see Fig. 4A which depicts the structure of SaXPD (Protein Data Bank ID 3CRV) solved by the Tainer lab.

The XPD crystal structures revealed two RecA-like helicase domains, present in all SF1 and SF2 helicases [64]. The two motor domains have a central interface in which ATP is presumed to be bound and hydrolyzed. The motion of the two motor domains relative to one other during ATP binding and hydrolysis has been proposed to drive DNA translocation.

The three recently solved crystal structures of XPD confirmed the existence of a novel Fe-S domain, first identified biochemically in 2006 by the White lab [65]. Mutations of the Fe-S domain, including the conserved cysteines, in SaXPD abolished helicase activity and/or destabilized tertiary structure [61, 65], attesting to the structural importance of the Fe-S domain. Site-directed mutagenesis of the four conserved cysteines of the Fe-S cluster in the Rad3 (XPD) helicase from *Ferroplasma acidarmanus* (FacRad3) revealed that the integrity of the domain is required for the proper folding and structural stability of the auxiliary domain and is important for coupling ATP hydrolysis to unidirectional translocation of helicase [66]. Based on their findings, Pugh et al. suggested that the Fe-S cluster domain is involved in the recognition of the ssDNA-dsDNA junction and positions the helicase in an orientation that supports duplex unwinding.

An important question is whether the Fe-S cluster is a purely structural domain or a specialized sensor of specific DNA damage? Based on the XPD crystal structures, it was proposed that the FeS domain plays a structural role to stabilize the enzyme and to serve as a wedge to physically separate the DNA duplex strands. In addition, the FeS domain could be a detector for different types of bulky DNA damage. DNA glycosylases [67–69] and a primase [70] contain Fe-S domains and may use their redox properties to recognize damaged bases. The presence of Fe-S cluster domains in FANCJ-like helicases is provocative because this particular class of DNA helicases has roles in DNA repair and the response to replicational stress. Since DNA helicases are among the first proteins to encounter DNA damage [71], it is of interest that the FANCJ-like helicases have a putative DNA damage sensor built into their machinery that utilizes redox potential. Biochemical evidence suggests that the conserved Fe-S domain in FANCJ plays a role in unwinding damaged DNA substrates [72]; moreover, *FANCJ* and *XPD* mutations in the Fe-S domain have clinical significance. Although the precise molecular function of the Fe-S domain is not fully understood, genetic evidence indicates the importance of the Fe-S domain in XPD. Disruption of the Fe-S cluster in yeast Rad3 (XPD ortholog in *Saccharomyces cerevisiae*) [65] or Rad15 (XPD ortholog in *Schizosaccharomyces pombe*) [73] causes UV sensitivity.

The Arch domain, named by its arch-shaped conformation, sits above the central cleft between the two motor domains, making contacts with both. Together with the Fe-S domain, the Arch domain forms an enclosed tunnel. Positively charged residues from the Arch domain and aromatic side chains from amino acids of the Fe-S domain suggest a topography that would provide a tunnel for ssDNA.

The XPD helicase structures provided new insight to the molecular basis for clinically relevant mutations in XPD as well as FANCJ. Mutations genetically linked to FA Complementation group J patients, as well as FANCJ sequence changes found in women with breast cancer are found mainly in the conserved helicase core domain (Fig. 2). A homology model of FANCJ based on the SaXPD structure (Protein Data Bank ID 3CRV) demonstrates that the two RecA motor domains and the accessory Fe-S and Arch domains are present in FANCJ (Fig. 4B). However, an additional autonomously folded domain corresponding to a unique sequence of FANCJ between motif I and Ia in RecA motor domain 1 was evident from the three-dimensional modeling using either the Swiss-model (Fig. 4B) or Biodesigner programs. Structural determination of FANCJ should help to define this and other domains (e.g., BRCA1-interacting domain) and provide an additional understanding of the disease causing mutations. Furthermore, biochemical studies that elucidate the mechanism of DNA translocation and unwinding by FANCJ-like helicases will help to determine both the unifying and unique properties of these enzymes that are important for their cellular roles. For example, XPD is required for NER whereas FANCJ is necessary for ICL repair. Understanding how their structural domains mediate DNA and protein interactions will help us to realize how the proteins perform their repair functions.

E. coli DinG Has a Similar DNA Substrate Preference as FANCJ

The newly found appreciation for the Fe-S domain in XPD and FANCJ has ignited an interest in the molecular mechanisms of FANCJ-like helicases. A DNA damage-inducible bacterial helicase known as DinG is a member of the FANCJ-like helicase family and also contains the conserved Fe-S cluster domain [65, 74]. Although DinG is not essential for viability, DinG expression is upregulated as a component of the SOS response in *E. coli*, and deletion or overexpression of *dinG* results in a slight reduction of UV resistance [75]. Voloshin et al. characterized purified recombinant DinG and showed that it is a highly active DNA dependent ATPase with a strong preference for ssDNA as the effector and exists as a monomer in solution [75]. Using forked duplex substrates with a synthetic linkage in the single-stranded tail adjacent to the duplex, DinG was shown to display a 5' to 3' helicase directionality [75]. Further analysis of the DNA unwinding activity of DinG revealed that this helicase displays a substrate preference very similar to FANCJ [74]. Similar to what was observed for FANCJ [15], DinG requires a 5' ssDNA tail for the helicase to unwind a conventional Watson-Crick duplex substrate; however, DinG unwinds D-loop substrates that lack any pre-exisiting ssDNA tail [74], a property also found for FANCJ [15]. Interestingly, although DinG and FANCJ efficiently unwind the D-loop, an early intermediate of HR repair, neither helicase unwinds the four-stranded Holliday Junction structure [15, 74], another key intermediate of HR that appears later in the pathway. The remarkable similarity in DNA substrate preference of FANCJ and DinG suggests that conserved sequence elements within the helicase core domain such as the Fe-S cluster play a prominent role in DNA substrate recognition/loading and possibly unidirectional translocation, a notion that is consistent with the study of FacRad3 [66] (discussed below).

Structural Features that Determine the Unique Aspects of SF2 FANCJ-like Helicases

An issue pertaining to the mechanism of helicases and nucleic acid translocases that remains to be resolved is the structural basis for different translocation polarities of helicases (for review, see [64]). This is an important question since the DNA translocation and unwinding directionality of a given helicase is a critical aspect of helicase function in processes (DNA replication, repair, transcription) that operate on nucleic acid strand(s) that by their very nature have a defined polarity dictated by their sugar phosphate backbone. The SF2 helicases are largely 3' to 5' helicases with the exception of the FANCJ/XPD family, which translocate and unwind dsDNA with a 5' to 3' directionality. The sequence conservation of the helicase motifs among SF2 proteins, and the presence of structurally related RecA domains 1 and 2 suggest that helicases such as XPD bind ssDNA along the top of the two RecA domains in a manner similar to other SF2 helicases, including 3' to 5' helicases (e.g., Hel308), whose structure has been solved with ssDNA [76] and motor domains can be overlaid with that of XPD [62]. Structural determination of the Fe-S and Arch domains in XPD has led to a new appreciation of the importance of these domains in the mechanism of DNA unwinding. The collective structural and biochemical evidence indicate that the conserved Fe-S domain is essential for XPD helicase activity and suggests that it serves as a recognition domain for the ssDNA-dsDNA junction, acting as the wedge for separation of complementary strands [61–63, 65]. It was proposed that the Arch and Fe-S domains, by their location adjacent next to the RecA motor domain 1, may serve to channel the 5' ssDNA end across the top of the two RecA motor domains. What remains to be determined is if and how these domains, acting in concert with the ATP hydrolysis dependent conformational changes of the two RecA core domains, determine the unidirectional movement of the helicase on ssDNA or ssDNA flanked by dsDNA. However, evidence in support of this notion is garnered from biochemical studies of FacRad3 and engineered Fe-S domain mutants which demonstrate that the integrity of the Fe-S domain is essential for coupling ATP hydrolysis to ssDNA translocation, and that the Fe-S domain targets the FacRad3 helicase to the ssDNA-dsDNA junction [66].

The structural features of the conserved helicase core and accessory domains revealed by the analyses of XPD crystals raise another important issue regarding substrate specificity of members of this family compared to other SF2 helicases. As discussed above, the FANCJ-like helicases have a highly selective substrate specificity requirement for a preexisting 5' ssDNA tail in order to load and unwind the adjacent duplex region [15, 74]. This substrate specificity would be consistent with a threading mechanism for helicase loading, perhaps through a central hole formed by RecA motor domain 1 with the adjacent Fe-S and Arch domains characteristic of XPD [61–63]. In contrast, SF2 3' to 5' RecQ helicases (*E. coli* RecQ [77] and human RECQ1 (Protein Data Bank ID 2V1X)) have the two conserved RecA motor domains but lack the Fe-S or Arch domains. Moreover, the RecQ helicases are much more promiscuous in terms of their substrate specificity [16]. WRN [78], BLM [79], and RECQ1 [80] helicases all have the ability to unwind 5' flap substrates that lack a preexisting 3' ssDNA tail. In fact, WRN unwinds a synthetic replication fork structure which lacks preexisting ssDNA altogether [78]. Another feature of the RecQ helicases that distinguishes them from the FANCJ-like helicases is their ability to branch-migrate Holliday Junction structures [16]. It is of great interest to understand the structural and mechanistic basis for the differences in substrate specificity between helicases belonging to the two families of SF2 helicases. Notably, the crystal structures of *E. coli* RecQ [77] and human RECQ1 (Protein Data Bank ID 2V1X) reveal the presence of a winged helix domain that has been implicated in DNA binding and protein interactions. Co-crystals of RecQ- and FANCJ-like helicases with DNA will likely provide new insights to the differences in helicase directionality and substrate specificity of these sequence related but structurally distinct SF2 families.

Future Perspectives

It is clear from the studies of DNA helicases sharing sequence similarity with FANCJ in the helicase core domain that these proteins play important roles in the maintenance of genomic stability and DNA repair. The wealth of biochemical and genetic information on XPD, now joined by structural insights, should provide a model for the study of related helicases. In addition to DNA repair of bulky adducts by nucleotide excision repair and ICLs by a specialized recombinational repair pathway, it seems likely that at least some members of the FANCJ-like helicase family play an important role in G-quadruplex DNA metabolism that might influence the stability of G-rich tracts in the genome. Although a direct and precise role of a FANCJ-like helicase in the replication or repair of a sub-telomeric structure remains to be shown, this will likely become an interesting area of study. The sister chromatid cohesion defects in *Saccharomyces cerevi-*

siae chl1 mutants, *DDX11* mutant mice, and ChlR1-depleted human cells, as well as the telomere shortening in human or mouse cells containing mutations in the *DDX11* and *rtel* genes, respectively, may be a consequence of aberrant mitotic chromosome structures due to genomic sequences that form alternate DNA arrangements such as G4 structures. In addition to specialized structures like the telomeres, processes such as sister chromatid cohesion are essential for chromosomal stability and play critical roles in the prevention of cellular aging and cancer. Now a new era of challenges is ushered in to define the roles of FANCJ-like helicases in pathways that prevent deleterious events at the DNA level associated with mutations, genomic instability, and disease.

Acknowledgements. This work was supported by the Intramural Research program of the NIH, National Institute on Aging and the Fanconi Anemia Research Fund (RMB). We wish to thank Drs. L. Fan and J. Tainer for kindly providing their XPD crystal structure and Elsevier for permission to reproduce their data in Fig. 4A. We thank Dr. Deborah L. Croteau (NIA, NIH) and Dr. Wei Yang (NIDDK, NIH) for critical reading of the manuscript.

1 Cantor, S. B., Bell, D.W., Ganesan, S., Kass, E. M., Drapkin, R., Grossman, S., Wahrer, D. C., Sgroi, D. C., Lane, W. S., Haber, D. A. and Livingston, D. M. (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 105, 149–160.
2 Levitus, M., Waisfisz, Q., Godthelp, B. C., de Vries, Y., Hussain, S., Wiegant, W. W., Elghalbzouri-Maghrani, E., Steltenpool, J., Rooimans, M. A., Pals, G., Arwert, F., Mathew, C. G., Zdzienicka, M. Z., Hiom, K., De Winter, J. P. and Joenje, H. (2005) The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. Nat. Genet. 37, 934–935.
3 Levran, O., Attwooll, C., Henry, R. T., Milton, K. L., Neveling, K., Rio, P., Batish, S. D., Kalb, R., Velleuer, E., Barral, S., Ott, J., Petrini, J., Schindler, D., Hanenberg, H. and Auerbach, A. D. (2005) The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. Nat. Genet. 37, 931–933.
4 Litman, R., Peng, M., Jin, Z., Zhang, F., Zhang, J., Powell, S., Andreassen, P. R. and Cantor, S. B. (2005) BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell 8, 255–265.
5 Kumaraswamy, E. and Shiekhattar, R. (2007) Activation of BRCA1/BRCA2-Associated Helicase BACH1 Is Required for Timely Progression through S Phase. Mol. Cell Biol. 27, 6733–6741.
6 Wu, Y., Shin-Ya, K. and Brosh, R. M., Jr. (2008) FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability. Mol. Cell Biol. 28, 4116–4128.
7 Bridge, W. L., Vandenberg, C. J., Franklin, R. J. and Hiom, K. (2005) The BRIP1 helicase functions independently of BRCA1 in the Fanconi anemia pathway for DNA crosslink repair. Nat. Genet. 37, 953–957.
8 Youds, J. L., Barber, L. J., Ward, J. D., Collis, S. J., O'Neil, N. J., Boulton, S. J. and Rose, A.M. (2008) DOG-1 is the Caenorhabditis elegans BRIP1/FANCJ homologue and functions in interstrand cross-link repair. Mol. Cell Biol. 28, 1470–1479.
9 Levitus, M., Joenje, H. and De Winter, J. P. (2006) The Fanconi anemia pathway of genomic maintenance. Cell Oncol. 28, 3–29.
10 Taniguchi, T. and D'Andrea, A. D. (2006) Molecular pathogenesis of Fanconi anemia: recent progress. Blood 107, 4223–4233.
11 Wang, W. (2007) Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat. Rev. Genet. 8, 735–748.
12 De, N. A., Tancredi, M., Lombardi, G., Flemma, C. C., Barbuti, S., Di, C. C., Sobhian, B., Bevilacqua, G., Drapkin, R. and Caligo, M. A. (2008) A novel breast cancer-associated BRIP1 (FANCJ/BACH1) germ-line mutation impairs protein stability and function. Clin. Cancer Res. 14, 4672–4680.
13 Seal, S., Thompson, D., Renwick, A., Elliott, A., Kelly, P., Barfoot, R., Chagtai, T., Jayatilake, H., Ahmed, M., Spanova, K., North, B., McGuffog, L., Evans, D. G., Eccles, D., Easton, D. F., Stratton, M.R. and Rahman, N. (2006) Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. Nat. Genet. 38, 1239–1241.
14 Cantor, S., Drapkin, R., Zhang, F., Lin, Y., Han, J., Pamidi, S. and Livingston, D. M. (2004) The BRCA1-associated protein BACH1 is a DNA helicase targeted by clinically relevant inactivating mutations. Proc. Natl. Acad. Sci. U. S. A 101, 2357–2362.
15 Gupta, R., Sharma, S., Sommers, J. A., Jin, Z., Cantor, S. B. and Brosh, R. M., Jr. (2005) Analysis of the DNA substrate specificity of the human BACH1 helicase associated with breast cancer. J. Biol. Chem. 280, 25450–25460.
16 Sharma, S., Doherty, K. M. and Brosh, R. M. Jr. (2006) Mechanisms of RecQ helicases in pathways of DNA metabolism and maintenance of genomic stability. Biochem. J. 398, 319–337.
17 Peng, M., Litman, R., Jin, Z., Fong, G. and Cantor, S. B. (2006) BACH1 is a DNA repair protein supporting BRCA1 damage response. Oncogene 25, 2245–2253.
18 Greenberg, R. A., Sobhian, B., Pathania, S., Cantor, S. B., Nakatani, Y. and Livingston, D. M. (2006) Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. Genes Dev. 20, 34–46.
19 Peng, M., Litman, R., Xie, J., Sharma, S., Brosh, R. M., Jr. and Cantor, S. B. (2007) The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. EMBO J. 26, 3238–3249.
20 Gupta, R., Sharma, S., Sommers, J. A., Kenny, M. K., Cantor, S. B. and Brosh, R. M., Jr. (2007) FANCJ (BACH1) helicase forms DNA damage inducible foci with replication protein A and interacts physically and functionally with the single-stranded DNA-binding protein. Blood 110, 2390–2398.
21 Cheung, I., Schertzer, M., Rose, A. and Lansdorp, P. M. (2002) Disruption of dog-1 in Caenorhabditis elegans triggers deletions upstream of guanine-rich DNA. Nat. Genet. 31, 405–409.
22 Eddy, J. and Maizels, N. (2006) Gene function correlates with potential for G4 DNA formation in the human genome. Nucleic Acids Res. 34, 3887–3896.
23 Maizels, N. (2006) Dynamic roles for G4 DNA in the biology of eukaryotic cells. Nat. Struct. Mol. Biol. 13, 1055–1059.
24 Youds, J. L., O'Neil, N. J. and Rose, A. M. (2006) Homologous recombination is required for genome stability in the absence of DOG-1 in Caenorhabditis elegans. Genetics 173, 697–708.
25 Kruisselbrink, E., Guryev, V., Brouwer, K., Pontier, D. B., Cuppen, E. and Tijsterman, M. (2008) Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-defective C. elegans. Curr. Biol. 18, 900–905.
26 Zhao, Y., Tarailo-Graovac, M., O'Neil, N. J. and Rose, A. M. (2008) Spectrum of mutational events in the absence of DOG-1/FANCJ in Caenorhabditis elegans. DNA Repair (Amst) 7, 1846–1854.
27 Gerring, S. L., Spencer, F. and Hieter, P. (1990) The CHL 1 (CTF 1) gene product of Saccharomyces cerevisiae is important for chromosome transmission and normal cell cycle progression in G2/M. EMBO J. 9, 4347–4358.
28 Haber, J. E. (1974) Bisexual mating behavior in a diploid of Saccharomyces cerevisiae: evidence for genetically controlled

non-random chromosome loss during vegetative growth. Genetics 78, 843–858.
29 Liras, P., McCusker, J., Mascioli, S. and Haber, J. E. (1978) Characterization of a mutation in yeast causing nonrandom chromosome loss during mitosis. Genetics 88, 651–671.
30 Spencer, F., Gerring, S. L., Connelly, C. and Hieter, P. (1990) Mitotic chromosome transmission fidelity mutants in Saccharomyces cerevisiae. Genetics 124, 237–249.
31 Swanson, C., Saintigny, Y., Emond, M. J. and Monnat, R. J., Jr. (2004) The Werner syndrome protein has separable recombination and survival functions. DNA Repair (Amst) 3, 475–482.
32 Holloway, L. (2000) CHL1 is a nuclear protein with an essential ATP binding site that exhibits a size-dependent effect on chromosome segregation. Nucleic Acids Res. 28, 3056–3064.
33 Skibbens, R. V. (2004) Chl1p, a DNA helicase-like protein in budding yeast, functions in sister-chromatid cohesion. Genetics 166, 33–42.
34 Skibbens, R. V. (2005) Unzipped and loaded: the role of DNA helicases and RFC clamp-loading complexes in sister chromatid cohesion. J. Cell Biol. 169, 841–846.
35 Petronczki, M., Chwalla, B., Siomos, M. F., Yokobayashi, S., Helmhart, W., Deutschbauer, A. M., Davis, R. W., Watanabe, Y. and Nasmyth, K. (2004) Sister-chromatid cohesion mediated by the alternative RF-CCtf18/Dcc1/Ctf8, the helicase Chl1 and the polymerase-alpha-associated protein Ctf4 is essential for chromatid disjunction during meiosis II. J. Cell Sci. 117, 3547–3559.
36 Ansbach, A. B., Noguchi, C., Klansek, I. W., Heidlebaugh, M., Nakamura, T. M. and Noguchi, E. (2008) RFCCtf18 and the Swi1-Swi3 complex function in separate and redundant pathways required for the stabilization of replication forks to facilitate sister chromatid cohesion in Schizosaccharomyces pombe. Mol. Biol. Cell 19, 595–607.
37 Mayer, M. L., Pot, I., Chang, M., Xu, H., Aneliunas, V., Kwok, T., Newitt, R., Aebersold, R., Boone, C., Brown, G. W. and Hieter, P. (2004) Identification of protein complexes required for efficient sister chromatid cohesion. Mol. Biol. Cell 15, 1736–1745.
38 Amann, J., Valentine, M., Kidd, V. J. and Lahti, J. M. (1996) Localization of chi1-related helicase genes to human chromosome regions 12p11 and 12p13: similarity between parts of these genes and conserved human telomeric-associated DNA. Genomics 32, 260–265.
39 Amann, J., Kidd, V. J. and Lahti, J. M. (1997) Characterization of putative human homologues of the yeast chromosome transmission fidelity gene, CHL1. J. Biol. Chem. 272, 3823–3832.
40 Vasa-Nicotera, M., Brouilette, S., Mangino, M., Thompson, J. R., Braund, P., Clemitson, J. R., Mason, A., Bodycote, C. L., Raleigh, S. M., Louis, E. and Samani, N. J. (2004) Mapping of a Major Locus that Determines Telomere Length in Humans. Am. J. Hum. Genet. 76**,** 147–151.
41 Parish, J. L., Rosa, J., Wang, X., Lahti, J. M., Doxsey, S. J. and Androphy, E. J. (2006) The DNA helicase ChlR1 is required for sister chromatid cohesion in mammalian cells. J. Cell Sci. 119, 4857–4865.
42 Inoue, A., Li, T., Roby, S. K., Valentine, M. B., Inoue, M., Boyd, K., Kidd, V. J. and Lahti, J. M. (2007) Loss of ChlR1 helicase in mouse causes lethality due to the accumulation of aneuploid cells generated by cohesion defects and placental malformation. Cell Cycle 6, 1646–1654.
43 Parish, J. L., Bean, A. M., Park, R. B. and Androphy, E. J. (2006) ChlR1 is required for loading papillomavirus E2 onto mitotic chromosomes and viral genome maintenance. Mol. Cell 24, 867–876.
44 Hirota, Y. and Lahti, J. M. (2000) Characterization of the enzymatic activity of hChlR1, a novel human DNA helicase. Nucleic Acids Res. 28, 917–924.
45 Farina, A., Shin, J. H., Kim, D. H., Bermudez, V. P., Kelman, Z., Seo, Y. S. and Hurwitz, J. (2008) Studies with the human cohesin establishment factor, ChlR1. Association of ChlR1 with Ctf18-RFC and Fen1. J. Biol. Chem. 283, 20925–20936.
46 Skibbens, R. V. (2008) Cell biology of cancer: BRCA1 and sister chromatid pairing reactions? Cell Cycle 7, 449–452.
47 Ding, H., Schertzer, M., Wu, X., Gertsenstein, M., Selig, S., Kammori, M., Pourvali, R., Poon, S., Vulto, I., Chavez, E., Tam, P. P., Nagy, A. and Lansdorp, P. M. (2004) Regulation of murine telomere length by Rtel: an essential gene encoding a helicase-like protein. Cell 117, 873–886.
48 Wu, X., Sandhu, S. and Ding, H. (2007) Establishment of conditional knockout alleles for the gene encoding the regulator of telomere length (RTEL). Genesis. 45, 788–792.
49 Barber, L. J., Youds, J. L., Ward, J. D., McIlwraith, M. J., O'Neil, N. J., Petalcorin, M. I. R., Martin, J. S., Collis, S. J., Cantor, S. B., Auclair, M., Tissenbaum, H., West, S. C., Rose, A. M. and Boulton, S. J. (2008) RTEL1 maintains genomic stability by suppressing homologous recombination. Cell 135, 261–271.
50 Friedberg, E. C., Bardwell, A. J., Bardwell, L., Feaver, W. J., Kornberg, R. D., Svejstrup, J. Q., Tomkinson, A. E. and Wang, Z. (1995) Nucleotide excision repair in the yeast Saccharomyces cerevisiae: its relationship to specialized mitotic recombination and RNA polymerase II basal transcription. Philos. Trans. R. Soc. Lond B Biol. Sci. 347, 63–68.
51 Hoeijmakers, J. H., Egly, J. M. and Vermeulen, W. (1996) TFIIH: a key component in multiple DNA transactions. Curr. Opin. Genet. Dev. 6, 26–33.
52 Lehmann, A. R. (2001) The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases. Genes Dev. 15, 15–23.
53 Lehmann, A. R. (2003) DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Biochimie 85, 1101–1111.
54 Cleaver, J. E. (1968) Defective repair replication of DNA in xeroderma pigmentosum. Nature 218, 652–656.
55 Broughton, B. C., Lehmann, A. R., Harcourt, S. A., Arlett, C. F., Sarasin, A., Kleijer, W. J., Beemer, F. A., Nairn, R. and Mitchell, D. L. (1990) Relationship between pyrimidine dimers, 6–4 photoproducts, repair synthesis and cell survival: studies using cells from patients with trichothiodystrophy. Mutat. Res. 235, 33–40.
56 Chen, J. and Suter, B. (2003) Xpd, a structural bridge and a functional link. Cell Cycle 2, 503–506.
57 Schaeffer, L., Moncollin, V., Roy, R., Staub, A., Mezzina, M., Sarasin, A., Weeda, G., Hoeijmakers, J. H. and Egly, J. M. (1994) The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. EMBO J. 13, 2388–2392.
58 Winkler, G. S., Araujo, S. J., Fiedler, U., Vermeulen, W., Coin, F., Egly, J. M., Hoeijmakers, J. H., Wood, R. D., Timmers, H. T. and Weeda, G. (2000) TFIIH with inactive XPD helicase functions in transcription initiation but is defective in DNA repair. J. Biol. Chem. 275, 4258–4266.
59 Coin, F., Oksenych, V. and Egly, J. M. (2007) Distinct roles for the XPB/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair. Mol. Cell 26, 245–256.
60 Dubaele, S., Proietti De, S. L., Bienstock, R. J., Keriel, A., Stefanini, M., Van, H. B. and Egly, J. M. (2003) Basal transcription defect discriminates between xeroderma pigmentosum and trichothiodystrophy in XPD patients. Mol. Cell 11, 1635–1646.
61 Fan, L., Fuss, J. O., Cheng, Q. J., Arvai, A. S., Hammel, M., Roberts, V. A., Cooper, P. K. and Tainer, J. A. (2008) XPD helicase structures and activities: insights into the cancer and aging phenotypes from XPD mutations. Cell 133, 789–800.
62 Liu, H., Rudolf, J., Johnson, K. A., McMahon, S. A., Oke, M., Carter, L., McRobbie, A. M., Brown, S. E., Naismith, J. H. and White, M. F. (2008) Structure of the DNA repair helicase XPD. Cell 133, 801–812.
63 Wolski, S. C., Kuper, J., Hanzelmann, P., Truglio, J. J., Croteau, D. L., Van, H. B. and Kisker, C. (2008) Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD. PLoS. Biol. 6, e149

64 Singleton, M. R., Dillingham, M. S. and Wigley, D. B. (2007) Structure and mechanism of helicases and nucleic acid translocases. Annu. Rev. Biochem. 76, 23–50.
65 Rudolf, J., Makrantoni, V., Ingledew, W. J., Stark, M. J. and White, M. F. (2006) The DNA repair Helicases XPD and FANCJ have essential Iron-Sulfur domains. Mol. Cell 23, 801–808.
66 Pugh, R. A., Honda, M., Leesley, H., Thomas, A., Lin, Y., Nilges, M. J., Cann, I. K. and Spies, M. (2008) The iron-containing domain is essential in Rad3 helicases for coupling of ATP hydrolysis to DNA translocation and for targeting the helicase to the single-stranded DNA-double-stranded DNA junction. J. Biol. Chem. 283, 1732–1743.
67 Cunningham, R. P., Asahara, H., Bank, J. F., Scholes, C. P., Salerno, J. C., Surerus, K., Munck, E., McCracken, J., Peisach, J. and Emptage, M.H. (1989) Endonuclease III is an iron-sulfur protein. Biochemistry 28, 4450–4455.
68 Guan, Y., Manuel, R. C., Arvai, A. S., Parikh, S. S., Mol, C. D., Miller, J. H., Lloyd, S. and Tainer, J. A. (1998) MutY catalytic core, mutant and bound adenine structures define specificity for DNA repair enzyme superfamily. Nat. Struct. Biol. 5, 1058–1064.
69 Kuo, C. F., McRee, D. E., Fisher, C. L., O'Handley, S. F., Cunningham, R. P. and Tainer, J. A. (1992) Atomic structure of the DNA repair [4Fe-4S] enzyme endonuclease III. Science 258, 434–440.
70 Weiner, B. E., Huang, H., Dattilo, B. M., Nilges, M. J., Fanning, E. and Chazin, W. J. (2007) An iron-sulfur cluster in the C-terminal domain of the p58 subunit of human DNA primase. J. Biol. Chem. 282, 33444–33451.
71 Villani, G. and Tanguy, L. G. (2000) Interactions of DNA helicases with damaged DNA: possible biological consequences. J. Biol. Chem. 275, 33185–33188.
72 Gupta, R., Sharma, S., Doherty, K. M., Sommers, J. A., Cantor, S. B. and Brosh, R. M., Jr. (2006) Inhibition of BACH1 (FANCJ) helicase by backbone discontinuity is overcome by increased motor ATPase or length of loading strand. Nucleic Acids Res. 34, 6673–6683.
73 Berneburg, M., Clingen, P. H., Harcourt, S. A., Lowe, J. E., Taylor, E. M., Green, M.H., Krutmann, J., Arlett, C. F. and Lehmann, A. R. (2000) The cancer-free phenotype in trichothiodystrophy is unrelated to its repair defect. Cancer Res. 60, 431–438.
74 Voloshin, O. N. and Camerini-Otero, R. D. (2007) The DinG protein from Escherichia coli is a structure-specific helicase. J. Biol. Chem. 282, 18437–18447.
75 Voloshin, O. N., Vanevski, F., Khil, P. P. and Camerini-Otero, R. D. (2003) Characterization of the DNA damage-inducible helicase DinG from Escherichia coli. J. Biol. Chem. 278, 28284–28293.
76 Buttner, K., Nehring, S. and Hopfner, K. P. (2007) Structural basis for DNA duplex separation by a superfamily-2 helicase. Nat. Struct. Mol. Biol. 14, 647–652.
77 Bernstein, D. A., Zittel, M. C. and Keck, J. L. (2003) High-resolution structure of the E.coli RecQ helicase catalytic core. EMBO J. 22, 4910–4921.
78 Brosh, R. M. Jr.., Waheed, J. and Sommers, J. A. (2002) Biochemical characterization of the DNA substrate specificity of Werner syndrome helicase. J. Biol. Chem. 277, 23236–23245.
79 Sharma, S., Sommers, J. A., Wu, L., Bohr, V. A., Hickson, I. D. and Brosh, R. M., Jr. (2003) Stimulation of flap endonuclease-1 by the Bloom's syndrome protein. J. Biol. Chem. 279, 9847–9856.
80 Sharma, S., Sommers, J. A., Choudhary, S., Faulkner, J. K., Cui, S., Andreoli, L., Muzzolini, L., Vindigni, A. and Brosh, R. M., Jr. (2005) Biochemical analysis of the DNA unwinding and strand annealing activities catalyzed by human RECQ1. J. Biol. Chem. 280, 38072–38084.