SURVEY AND SUMMARY

DNA helicase and helicase–nuclease enzymes with a conserved iron–sulfur cluster

Yuliang Wu[1],* and Robert M. Brosh Jr[2],*

[1]Department of Biochemistry, University of Saskatchewan, Health Sciences Building, Saskatoon, Saskatchewan, S7N 5E5, Canada and [2]Laboratory of Molecular Gerontology, National Institute on Aging, NIH, NIH Biomedical Research Center, Baltimore, MD 21224, USA

ABSTRACT

Conserved Iron–Sulfur (Fe–S) clusters are found in a growing family of metalloproteins that are implicated in prokaryotic and eukaryotic DNA replication and repair. Among these are DNA helicase and helicase–nuclease enzymes that preserve chromosomal stability and are genetically linked to diseases characterized by DNA repair defects and/or a poor response to replication stress. Insight to the structural and functional importance of the conserved Fe–S domain in DNA helicases has been gleaned from structural studies of the purified proteins and characterization of Fe–S cluster site-directed mutants. In this review, we will provide a current perspective of what is known about the Fe–S cluster helicases, with an emphasis on how the conserved redox active domain may facilitate mechanistic aspects of helicase function. We will discuss testable models for how the conserved Fe–S cluster might operate in helicase and helicase–nuclease enzymes to conduct their specialized functions that help to preserve the integrity of the genome.

DNA METABOLIZING PROTEINS WITH THE CONSERVED IRON–SULFUR CLUSTER

Iron–Sulfur (Fe–S) clusters have been identified in a diverse group of proteins with a wide range of biological functions such as transcription regulation (e.g. SoxR, Elp3), spore photoproduct lyase, electron transport in the respiratory complexes of bacteria and mitochondria, photosystems, carbohydrate metabolism (e.g. aconitase) and biotin synthesis (e.g. S-adenosyl L-methionine biotin synthase) (1,2). Elaborate mechanisms for biogenesis of Fe–S clusters have been characterized (3–5). The growing number of human diseases with defects in Fe–S cluster assembly or function suggests that proteins with the Fe–S cluster are tailored for highly specialized and essential cellular duties (6,7). To begin, we will briefly discuss DNA repair glycosylases and the eukaryotic primase that contain the conserved Fe–S cluster, which provides a background to address the importance of the conserved motif for DNA helicases and helicase–nucleases. We will then focus our discussion on structural, mechanistic and biological aspects of DNA helicases and helicase–nucleases enzymes with conserved Fe–S clusters to gain a better appreciation of their specialization and significance. Redox activity of the Fe–S cluster is proposed to play an important role in the biochemical and cellular functions of this special family of DNA metabolic enzymes.

DNA GLYCOSYLASES

A 4Fe–4S cluster was first discovered in *Escherichia coli* endonuclease III, a glycosylase, which removes oxidized pyrimidines from duplex DNA (8). A number of additional base excision repair (BER) glycosylases, including an adenine and weak guanine glycosylase known as MutY (9,10) as well as a family of uracil DNA glycosylases (11) were subsequently identified with the conserved 4Fe–4S cluster [for review, see (12)]. Given the unique importance of DNA glycosylases as initiators of BER that cleave the glycosidic bond between damaged base and sugar, researchers have considered it highly relevant to characterize the structural and functional significance of the conserved Fe–S cluster in this class of enzymes, which display a wide range of substrate specificity. Biochemical and structural studies of Fe–S cluster glycosylases suggest that the domain containing the cluster is involved in DNA binding, and the interaction

of the Fe–S cluster domain with DNA directly affects efficiency of base lesion detection and removal (13,14). The 4Fe–4S clusters found in DNA glycosylases are vulnerable to oxidation when the domain contacts DNA, consistent with the redox potential of the Fe–S cluster. It has been proposed that the redox behavior of DNA glycosylases may aid this class of proteins to scan the DNA duplex for damaged bases. This area of research continues to be a highly debated one since it is of considerable interest to understand how DNA repair machinery efficiently locates sites of damage over the millions of base pairs in the human genome. Furthermore, clinically relevant variants of DNA glycosylases may have altered Fe–S domain structure or activity. For example, mutations in the gene encoding the human MutY homolog that are associated with colorectal cancer were identified that may perturb the proper function of the Fe–S cluster domain (15).

PRIMASES

Eukaryotic primases can initiate DNA synthesis by creating short RNA primers from individual ribonucleotides. Generally speaking, eukaryotic primases contain two subunits that are tightly associated with each other and DNA polymerase α. Studies from the Pellegrini (16) and Chazin (17) labs showed that the larger subunit of eukaryotic primase contain a conserved Fe–S domain whose integrity is required for primase to initiate cellular DNA replication. The conserved Fe–S domain is characterized by the appearance of four neighboring cysteine residues that are responsible for each chelating an Fe atom. The fact that the primase 4Fe–4S domain is required for enzymatic as well as biological activity suggests that the Fe–S cluster plays a critical role in its structure and function. It is conceivable that DNA primases like glycosylases and helicases, use the redox active Fe–S cluster domain to perform their DNA metabolic functions.

A high-resolution structure of a human primase domain, which harbors the conserved Fe–S cluster revealed that the Fe–S cluster is integral to the overall structure of the domain and helps to organize the protein surface to bind DNA (18). Furthermore, it was proposed that the Fe–S cluster may serve to facilitate DNA-mediated electron transport which could be important for the recognition of oxidized bases or other forms of DNA damage. Regulation of DNA primase activity through alteration of redox potential may occur during abnormal replication through regions of damaged DNA.

STRUCTURAL ASPECTS OF DNA HELICASES AND THEIR IMPORTANCE IN CELLULAR DNA METABOLISM

In order to appreciate the potential significance of the conserved Fe–S cluster in DNA helicases, it is helpful to have a general perspective of what is known about helicase structure, function and classification. Helicases are molecular motors that couple the energy of nucleoside triphosphate (NTP) hydrolysis to the unwinding and

remodeling of polynucleic acid structures (19,20). Helicases are involved in virtually all aspects of nucleic acid metabolism, including replication, DNA repair, recombination, transcription, chromosome segregation and telomere maintenance (20–24). Depending on its substrate, helicases can be classified as DNA or RNA unwinding enzymes, although some helicases can function on both DNA and RNA (23). DNA helicase-like proteins have been reported to act in a variety of DNA metabolic processes that include unwinding duplex or alternate (triplex, G-quadruplex) DNA, displacing protein bound to DNA, chromatin remodeling and strand annealing (25–28).

Based on their primary amino acid sequences, helicases are classified into four small families and two large families, superfamilies 1 and 2 (SF1 and SF2) (20). Fe–S clusters have been found in both superfamilies of DNA helicases (discussed below). Mutational and crystallographic studies have provided structural information on the spatial organization of the conserved motifs that form the helicase catalytic core. The crystal structures of SF1 helicases UvrD (29) and PcrA (30) and SF2 helicases, including RecG (31), NS3 (32), RecQ (33), VASA (34) and Hel308 (35,36), revealed that there are two universal RecA folds, which are essential for nucleic acid binding, NTP binding and hydrolysis and coupling of NTP hydrolysis to nucleic acid unwinding. Located at the interface between the two RecA-like domains are the seven signature helicase motifs, designated motifs I (or Walker A), Ia, II (or Walker B), III, IV, V and VI. In addition to these highly conserved seven helicase motifs, some helicases also harbor less conserved, family-typical domains, including a Q motif before motif I, two additional motifs (Ib and Ic) after Ia, an extra motifs IIIa after III, a motif IVa between IV and V and two more motifs (Va and Vb) between V and VI (20,37). Generally, motifs I, II, VI and Q are essential for ATP binding and hydrolysis, motifs Ia, Ib, IV and V are primarily responsible for DNA binding, and motif III and Va are important for coupling ATP hydrolysis to remodeling events such as unwinding (23,29).

It is likely that the accessory domains outside the RecA core domains are important for the specialized functions of each enzyme, thereby permitting helicases to be involved in diverse aspects of RNA and DNA metabolism. Recent studies of the SF2 XPD helicase revealed two unique domains (Arch and Fe–S) that separate this family of DNA helicases from others (next section).

DNA HELICASES WITH CONSERVED Fe–S CLUSTERS

The White lab first reported a conserved metal-binding domain in the XPD protein family of SF2 DNA helicases (38). Sequence alignment of a region between the Walker A and B boxes showed the presence of a conserved Fe–S cluster punctuated by four cysteine residues that was recognized in a variety of human, yeast and bacterial helicases (Figure 1). Clinically, relevant mutations in the Fe–S domain of XPD and FANCJ were shown to be
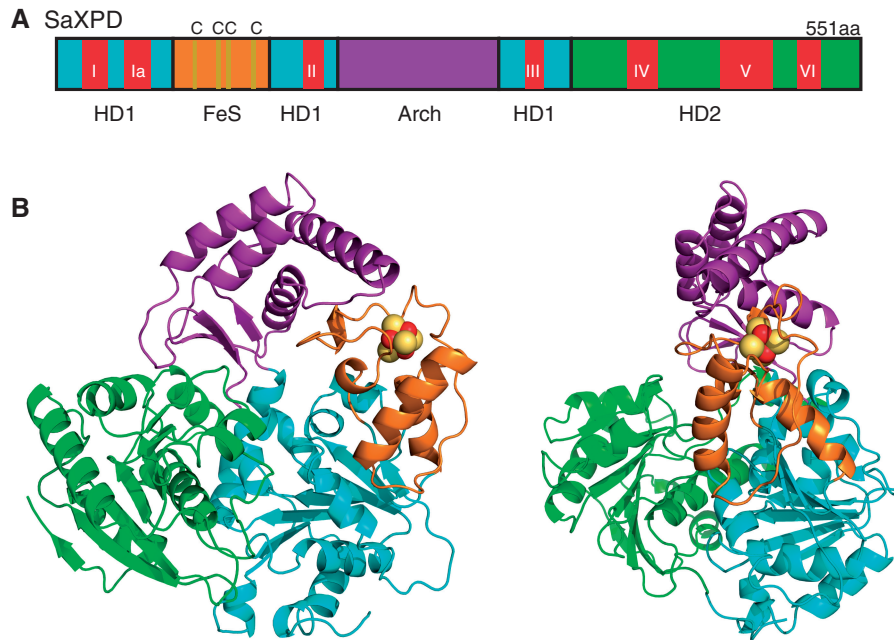
A

B

Figure 1. Fe–S cluster in the helicase domain of XPD/FANCJ family of DNA helicases. (**A**) Relative positions of the conserved Fe–S domain (yellow) are shown for the SF2 XPD/FANCJ family of DNA helicases. *Homo sapiens* (Hs) FANCJ, XPD, ChlR1, RTEL, *Ferroplasma acidarmanus* (Fac) XPD, *Saccharomyces cerevisiae* (Sc) Rad3 and *E. coli* (Ec) DinG are shown. Helicase domain is shown in purple. The BRCA1-binding domain of HsFANCJ is shown in green. The PCNA interaction motif (PIP) of HsRTEL is shown in blue. (**B**) Sequence alignment of the Fe–S domain helicases. The four conserved cysteine residues are highlighted in orange. The XPD amino acid substitution R112H genetically linked to trichothiodystrophy is indicated. The FANCJ amino acid substitutions M299I associated with breast cancer and A349P linked to Fanconi Anemia are shown. Amino acid positions corresponding to the beginning and end of the Fe–S cluster are indicated. Amino acids residing between conserved cysteines may not be shown due to space limitations.

genetically linked to the diseases trichothiodystrophy (TTD) and Fanconi Anemia (FA), respectively. The Fe–S domain helicases are emerging as a very important family of proteins with cellular roles in DNA repair, replication, transcription and maintenance of genomic stability.

XPD

XPD is a part of the transcription factor II H (TFIIH) core complex, which plays an important role in nucleotide excision repair (NER) as well as in basal transcription (39). TFIIH is a eleven-subunit complex composed of a core (XPG, XPB, p62, p52, p44, p34 and p8/TTDA) associated with CAK (Cdk7, Cyclin H and MAT1) through the XPD subunit (40). Mutations in the human *XPD* gene give rise to three different genetic disorders: xeroderma pigmentosum (XP), TTD and Cockayne's syndrome (CS). XPD is classified as a SF2 5′–3′ DNA helicase. Since XPD bridges the TFIIH core complex and CAK subcomplex, XPD protein–protein interactions are critical for the stability of the TFIIH complex. Mutations in ATPase and/or helicase motifs of XPD abolish TFIIH repair activity and DNA opening around

bulky DNA lesions that serve as substrates for NER. As part of the TFIIH core, XPD helicase activity is required in NER, but not for the initiation of transcription.

Unlike human XPD, archaeal XPD is a monomer in solution and has no known stable interactions with other proteins, which makes it amenable to biochemical and structural studies. The freshly purified recombinant *Sulfolobus acidocaldarius* (Sa) XPD that had been overexpressed in *E. coli* displayed a yellow–green color with a broad shoulder of absorbance between 360 and 550 nm, indicating it contained an Fe–S cluster (38). Further quantitative analysis demonstrated that wild-type SaXPD contains about three iron atoms per peptide. Site-directed mutagenesis implicated a set of four conserved cysteine residues comprising the Fe–S-cluster-binding site in yeast XPD (Rad3) (38). Further analysis showed that the Fe–S domain is not essential for SaXPD protein stability, the enzyme's ability to bind to single-stranded DNA, or its ATPase activity, but is required for helicase activity (38).

The three recently solved crystal structures of archaeal XPD confirmed the existence of a novel Fe–S domain (41–43). A structure of the apo-SaXPD catalytic core with a 4Fe–4S cluster is shown in Figure 2 (41). All solved XPD structures contained two Rad51/RecQ-like

Figure 2. Structure of the apo-XPD catalytic core from *Sulfolobus acidocaldarius*. (**A**) The four XPD catalytic core domains are depicted in boxes for helicase domain (HD) 1 (cyan), HD2 (green), 4FeS (orange) with cysteine (C) residues indicated and Arch (purple) domains. Conserved helicase motifs (red bars with white labels) are shown. (**B**) XPD catalytic core fold and domains (ribbons). HD1 (cyan) and HD2 (green) form the nucleotide-binding pocket. Front view (left) shows that an arch is formed by the insertion of 4FeS (orange) and Arch (purple) domains, into HD1. Side view (right) shows that HD2 protrudes from the flat box formed by HD1, 4FeS and Arch as well as the HD2 helix-loop-helix insertion (green). Figure was provided by Drs Jill Fuss and John Tainer (45).

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 (Figure 2). The conformational state of the Fe–S and Arch domains is intimately connected with conserved helicase motifs implicated in ATP binding and hydrolysis. It was proposed that the Fe–S domain forms a wedge shape with the nearby Arch domain to separate the DNA duplex as the enzyme translocates in an ATP-dependent manner. Mutations of the Fe–S domain, including the conserved cysteines, abolished SaXPD helicase activity and/or destabilized tertiary structure (38,41), 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* (FacXPD) 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 (44). Thus, the Fe–S cluster has dual functions to stabilize elements of protein secondary structure and target the helicase to the single-stranded/double-stranded DNA junction (44).

Quite recently, the first structure of XPD from *Thermoplasma acidophilium* (ta) in complex with a short DNA fragment was reported (Figure 3) (45). The path of the translocated DNA strand through the taXPD protein was suggested, providing a model for the mechanism of translocation by DNA helicases of the SF2B subfamily that contain a 4Fe–4S cluster. The taXPD–DNA crystal structure, combined with a mutational and biochemical
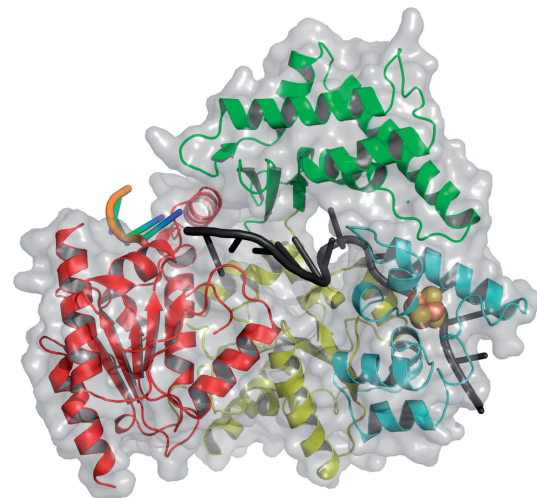
Figure 3. Structure of the *T. acidophilium* XPD–DNA complex. Overall structure of XPD, shown in a transparent surface representation, with the two RecA-like domains in yellow and red, the FeS cluster domain in cyan, and the arch domain in green. The 4Fe–4S cluster is shown by the spheres with orange (Fe atom) and yellow (Cys residue) colors. The DNA identified in the electron density is shown in orange. Combination of experimentally verified DNA is shown in orange with modeled DNA shown in gray. Figure was provided by Drs Jochen Kuper and Caroline Kisker (45).

analysis of taXPD, is significant because it revealed how the 5′ to 3′ directionality of translocation along DNA is achieved and suggests how the XPD enzyme might behave on relevant DNA substrates in NER. These conclusions

on how regulation of translocation polarity by an Fe–S domain helicase is achieved were further supported by another very recent study using proteolytic DNA and mutational analysis of taXPD (46). Collectively, these studies suggest that Fe–S domain helicases achieve a polarity of 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 was poorly understood how mutations in a single gene could lead to three different disorders until the crystal structure of archaeal XPD was solved, which greatly helped to explain the disease specificity of the individual mutations in human XPD (41–43). These studies suggested that XP-specific mutations would affect single-stranded DNA and ATP binding, which would disrupt XPD helicase function. The XP/CS phenotype mutations are likely to affect XPD conformation. Most TTD mutations affect XPD protein structural integrity, which in turn would disrupt protein–protein interactions of XPD with other subunits of TFIIH.

One of the most common mutations in TTD patients, amino acid substitution R112H, is localized in the Fe–S domain of XPD just before the first conserved cysteine residue (Figure 1). The R112H missense mutation has been shown to result in a complete loss of XPD helicase activity and a reduced basal transcription activity of the TFIIH complex (47). TTD patient cells that harbor the R112H variant also have reduced levels of TFIIH content (48). Given that amino acid R112 is localized in the Fe–S domain of XPD, it remains to be determined whether the R112H TTD mutation has an effect on XPD protein iron content, but it seems likely. Loss of iron content probably contributes to structural defects of the XPD mutant protein that abolish its helicase activity and interfere with TFIIH protein–protein interactions.

FANCJ

FANCJ was first identified as a protein that binds to the breast cancer C-terminal (BRCT) repeats of BRCA1, therefore named BACH1 (BRCA1-associated C-terminal helicase 1) (49), and subsequently renamed BRIP1 (BRCA1 interacting protein 1) because a transcription factor is named BACH1. BRIP1 was later identified as the gene mutated in the J complementation group of FA. Now, BACH1/BRIP1 is widely referred to as FANCJ (FA complementation group J). The identification of FANCJ mutations in early onset breast cancer patients (49,50) and FA Group J patients (51–53) implicates FANCJ as a tumor suppressor caretaker that functions in DNA double-strand break (DSB) and interstrand crosslink (ICL) repair. FANCJ is considered a low-to-moderate penetrance breast cancer susceptibility allele, and is associated with modest 2- to 3-fold increases in breast cancer risk (54), and accounts for 2% of FA patients (55). *FANCJ* is not only one of the genes linked to both FA and breast cancer, but also one of the few genes in the FA-BRCA pathways predicted to have direct roles in DNA metabolism (56).

FANCJ encodes a 1249 amino acid protein and contains seven conserved helicase motifs found in SF2 helicases. Biochemically, FANCJ has been demonstrated to be a bonafide DNA helicase that catalytically unwinds duplex DNA (57,58) and G-quadruplex structures (59,60) in an ATP hydrolysis-dependent manner. Studies support a role for FANCJ in homologous recombination repair of DNA ICLs and DSBs (53). FANCJ physically and functionally interacts with human Replication Protein A (61), MLH1 (62,63) and Bloom's syndrome helicase (BLM) (64), three key proteins in DNA repair and maintenance of genomic stability (61,64). The helicase activity of FANCJ is required for timely progression through S phase (65). FANCJ acts with TopBP1 in early DNA replication checkpoint control (66), suggesting that FANCJ has additional roles in the response to replication stress and operates in a parallel pathway to the classic FA pathway (67).

A pathogenic FANCJ-A349P amino acid substitution (Figure 1), which resides immediately adjacent to the fourth highly conserved cysteine of the Fe–S domain, was reported to result in severe phenotypic abnormalities, including intrauterine growth failure and death as a stillborn fetus with a gestational age of 22 weeks (52). Biochemical analysis of the purified recombinant FANCJ proteins demonstrated that FANCJ-A349P protein had a reduced iron content of one Fe atom per polypeptide compared to three Fe atoms in each wild-type FANCJ protein monomer (68). From a functional standpoint, the A349P substitution uncoupled ATP-dependent DNA translocase activity from the enzyme's ability to unwind DNA or displace proteins bound to DNA. To our knowledge, the effect of the A349P substitution on FANCJ catalytic activities is distinct from any other helicase disease mutation reported in the literature. Thus, the integrity of the Fe–S cluster is essential for the higher order catalytic functions of FANCJ. Importantly, these results demonstrate that the ability of FANCJ to couple DNA translocase activity to its other DNA metabolic functions is required for its roles in DNA repair. Furthermore, the *FANCJ-A349P* mutant allele exerted a dominant-negative effect on cellular resistance to agents that induce DNA damage or replication stress, confirming that FANCJ-A349P expression exerts deleterious effects on cellular phenotypes (69).

Another naturally occurring FANCJ mutation, M299I, has been identified in early-onset breast cancer patients without a family history of breast or ovarian cancer (57). Interestingly, the M299I substitution occurs immediately adjacent to the second conserved cysteine in the Fe–S domain of FANCJ (Figure 1). It is not known whether M299I affects FANCJ's metal-binding ability, although it seems likely that the Fe–S cluster remained intact since the purified recombinant FANCJ-M299I protein exhibited greater ATP hydrolysis and increased helicase activity than wild-type recombinant FANCJ *in vitro* (57,70). The increased motor ATPase activity of the FANCJ-M299I variant enabled the helicase to unwind backbone-modified DNA substrates in a more proficient manner (70), substantiating the gain of function exerted by the Fe–S domain mutation. How increased enzyme activity due to

perturbation of the Fe–S cluster or some other biochemical or structural effect exerted by the M299I mutation may contribute to cancer progression remains unexplained.

Although the role of XPD helicase in NER is reasonably well understood, the precise molecular functions of FANCJ in ICL repair or of ChlR1 in sister chromatid cohesion (next section) still remain a mystery. Further studies are necessary to understand how Fe–S domain helicases are uniquely equipped to perform their specialized functions that help cells cope with replication stress or DNA damage.

ChlR1

ChlR1 is a member of the FANCJ/XPD family of DNA helicases, capable of unwinding duplex DNA in a 5′–3′ direction in an ATP-dependent reaction (71,72). Recently, it was shown that hereditary recessive mutations in the *DDX11* gene encoding ChlR1 are responsible for a rare genetic disorder known as Warsaw breakage syndrome (WABS) (73). Studies of yeast CHL1 (74–76) and mammalian CHLR1 (77,78) demonstrate that the helicase is required for normal sister chromatid cohesion and the maintenance of genomic stability. The emerging evidence from yeast suggests that Chl1 participates in cohesion establishment, a process that is believed to be coupled with DNA replication. In *C. elegans*, CHL-1 is required for normal development, fertility and chromosomal stability (79). Interestingly, a role for *C. elegans* CHL-1 in G-quadruplex DNA metabolism was suggested by the observation that the number of G-tract deletions increased in worms that contained mutations in both *chl-1* and *dog-1* (79), which encodes a FANCJ-related helicase with a conserved Fe–S cluster [(80), see below]. Indeed, it was recently shown that ChlR1 efficiently unwinds anti-parallel G-quadruplex DNA (81).

Based on genetic evidence and the interaction of human ChlR1 with the structure-specific nuclease Flap Endonuclease 1 (FEN-1) (71), an enzyme that is implicated in replication fork lagging strand processing, a model was proposed that ChlR1 may function with nuclear factors such as FEN-1 to insure that replication through the pre-loaded cohesion ring proceeds smoothly. Although recent work suggests that ChlR1 facilitates sister chromatid cohesion by preserving heterochromatin organization and function in mammalian cells (82), the precise molecular functions of ChlR1 still remain to be clearly understood.

ChlR1 protein interactions with other factors associated with the DNA replication machinery [FEN-1, Proliferating cell nuclear antigen (PCNA), components of the RFC complex] (71) are likely required for ChlR1 helicase to perform its cellular functions. Although no evidence suggests that the Fe–S cluster integrity domain mediates protein interactions, its structural integrity is likely to directly or indirectly affect the ability of ChlR1 to collaborate with other proteins during DNA processing. The structural and functional importance of the conserved Fe–S cluster in ChlR1 has not yet been addressed experimentally. Given the location of disease-causing mutations immediately adjacent to the conserved cysteines in FANCJ, it will be important to examine sequence variants of ChlR1 and their effects on its biochemical and cellular functions.

DinG

Sequence analysis of the *E. coli* DNA damage inducible protein DinG predicted that the SF2 helicase would contain the conserved Fe–S cluster found in XPD and FANCJ helicases. This was biochemically confirmed by the Ding lab (83). The DinG helicase [4Fe–4S] cluster is redox-active with a midpoint potential close to that estimated for the *E. coli* cytoplasm. Reduction of the Fe–S cluster *in vitro* reversibly switches off DinG helicase activity. These observations led to speculation that the Fe–S cluster in DinG may act as a sensor of intracellular redox potential to modulate its helicase activity. Although ATPase and single-stranded DNA translocation were not examined, it would be of interest to know if reduction of the Fe–S cluster in DinG (or FANCJ) uncouples ATPase/translocase activity from DNA unwinding, similar to the behavior displayed by the FANCJ-A349P Fe–S domain mutant (68). The DinG Fe–S cluster was shown to be modified by nitric oxide (NO) (83), a free radical species that induces endogenous damage to DNA, proteins and lipids. These observations raise the possibility that DNA repair processes may be affected by NO and other forms of oxidative stress by their effects on Fe–S cluster proteins such as DNA helicases. Since the redox properties of Fe–S cluster domains in DNA-binding proteins, such as BER glycosylases (14,84) or certain transcription factors (e.g. SoxR) (85), can be altered when DNA is present, it will be of interest to assess the effect of DNA binding by DinG on its redox state. Future studies are likely to address as well if the eukaryotic Fe–S helicases are regulated by a redox mechanism.

A novel function for DinG to promote replication fork movement along transcribed DNA was recently described. DinG may serve as an accessory helicase to unwind R-loops or possibly displace the transcribing RNA polymerase, thereby enabling replication across transcription units to proceed at a normal rate (86). Although speculative at this time, it may be that Fe–S domain helicases represent a class of enzymes that have the ability to use their motor ATPase function to unwind non-conventional nucleic acid structures or displace proteins bound to DNA that interfere with normal genomic transactions. In support of this notion, human FANCJ was shown to unwind triplex (87) and G-quadruplex (59,60) DNA structures, and remove protein (e.g. Rad51) bound to single-stranded DNA (87). An archaeal XPD helicase is able to either displace or bypass a single-stranded DNA-binding protein in its path (88). Further studies may elucidate if the Fe–S domain itself helps to facilitate a specialized function of the helicase to unwind structured nucleic acids and/or strip proteins off DNA in addition
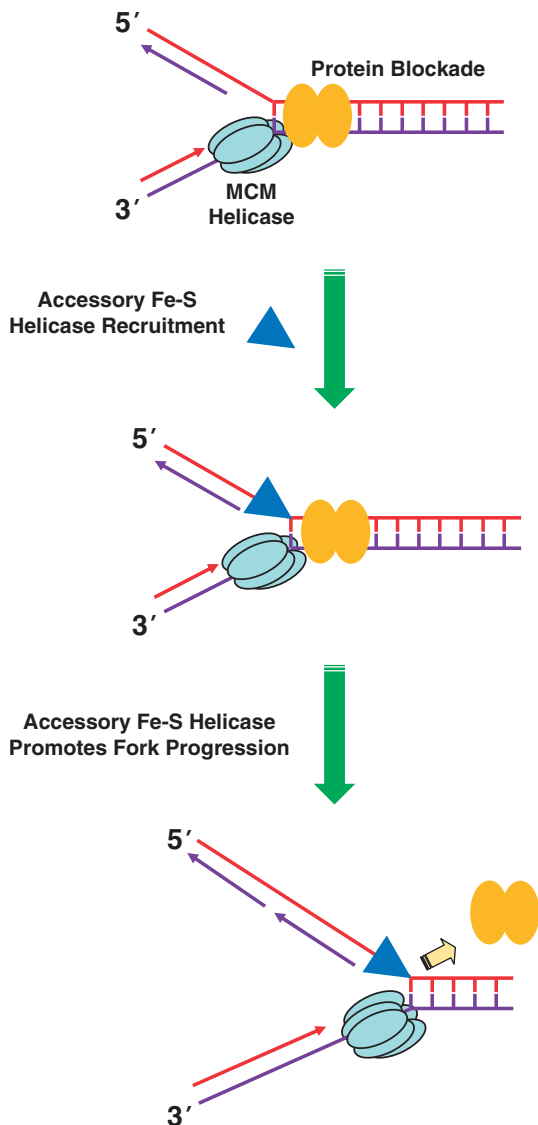
Figure 4. Model depicting the coordinate action of an accessory Fe–S cluster 5′–3′ helicase with the MCM 3′–5′ helicase to clear a protein blockade encountered by the eukaryotic MCM/ replication machinery. For simplicity, the replication machinery is not shown. Model is adapted by analogy from one proposed for *E. coli* DNA replication (90).

to targeting the helicase to single-stranded DNA–double-stranded DNA junctions, as shown for FacRad3 (XPD) by the Spies lab (44).

Recent work from the McGlynn lab suggests a model in which accessory helicases in *E. coli* sustain efficient DNA replication fork progression by displacing proteins bound to DNA (89,90). The 5′–3′ helicase activity characteristic of known eukaryotic Fe–S cluster helicases (FANCJ, XPD, ChlR1) would be of appropriate directionality for the Fe–S helicase to serve as an accessory helicase translocating on the opposite strand to that of the 3′–5′ MCM replicative helicase at the replication fork to displace proteins that impede fork progression (Figure 4).

DOG-1 AND RTEL

Mutations in *C. elegans dog-1*, an ortholog of mammalian FANCJ with the conserved Fe–S cluster, result in the accumulation of germline as well as somatic deletions in genes containing polyguanine tracts (80). Later work (91,92) confirmed these observations, leading researchers to speculate that DOG-1 helicase might efficiently unwind G-quadruplex DNA structures that impede cellular DNA replication and pose a source of genomic instability.

The murine *rtel* gene encodes a protein sharing sequence homology with DOG-1/FANCJ, including the conserved Fe–S cluster. *rtel* knockout mice were embryonic lethal and cells derived from these mice exhibited a rapid reduction in proliferative capacity upon differentiation, accompanied by an increased incidence of chromosomal abnormalities and telomere loss (93). The telomere loss characteristic of *rtel* mutant mice may be a direct consequence of defective DNA metabolism due to lack of specialized helicase action at the telomere end. In support of this, rtel-deficient mouse cells display elevated spontaneous fragile telomeres (94). Purified recombinant human RTEL can actively disrupt three-stranded D-loop recombination intermediates, which may be relevant to its putative anti-recombinase role (95). Moreover, since telomeric D-loops (T-loops) are suggested to stabilize or protect telomeres from de-stabilizing events (96,97), the biochemical activity of human RTEL is consistent with a predicted function of the Fe–S cluster helicase to unravel the hidden 3′-DNA end during cellular replication or repair.

Determination if RTEL associates with components of the shelterin complex and the functional consequences of the interaction should be revealing for finding clues to the potential involvement of the helicase in the prevention of a human telomere disease. A number of telomere diseases are known (98). Of particular interest is dyskeratosis congenita (DC), which is defective in the *DKC1* gene that encodes dyskerin (99), an RNA-binding protein that is a component of the telomerase complex (100). If indeed human RTEL interacts with the shelterin complex, it seems probable that Fe–S helicase may join the RecQ helicases WRN and BLM (101) as a specialized regulator of telomere maintenance.

HELICASE–NUCLEASE ENZYMES WITH CONSERVED Fe–S CLUSTERS

The Dillingham lab first reported a novel class of proteins with a nuclease domain that contains a conserved Fe–S cluster. This group includes the bacterial AddB nuclease and eukaryotic SF1 Dna2 helicase–nuclease (Figure 5). Four conserved cysteines flank the conserved RecB family nuclease domain. Given the importance of nucleases in DNA processing events associated with DNA repair and replication, the discovery of helicase–nuclease enzymes with conserved Fe–S clusters has sparked great interest in their structure and function.
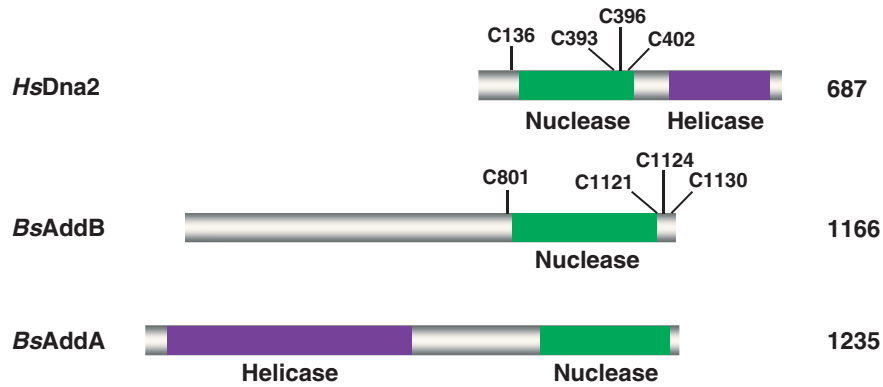
Figure 5. Fe–S cluster in the nuclease domain of eukaryotic Dna2 and AddB of the bacterial AddAB helicase–nuclease. Positions of the four conserved cysteine residues in the nuclease domain (green) are shown for human Dna2 and *Bacillus subtilis* AddB (102). The helicase domain is shown in purple. For Dna2, the nuclease and helicase domains reside in the same polypeptide. AddAB exists as a protein dimer with the Fe–S nuclease domain residing in AddB. AddA contains a second nuclease domain as well as a helicase domain.

AddAB

One of the newly identified DNA repair proteins with an Fe–S cluster is the AddB nuclease of the bacterial helicase–nuclease complex AddAB (102). AddAB-type complexes are found in Gram positive bacteria that lack the RecBCD-type enzyme complex. Like *E. coli* RecBCD, *Bacillus subtilis* AddAB is implicated in DSB repair through its ability to convert a DNA break into a 3′ single-stranded DNA overhang necessary for homologous recombinational repair. The dual nuclease reaction mechanism is regulated by a consensus DNA sequence element known as cross-over hotspot instigator (Chi). Conserved nuclease domains are found in the C-terminal regions of AddA and AddB subunits. The Dillingham lab discovered that the 5′–3′ nuclease domain of AddB contains an Fe–S cluster (102). The AddB Fe–S domain contains four conserved cysteine residues with the first cysteine residing N-terminal to the nuclease domain and the other three cysteines residing on the C-terminal side. Characterization of the Fe–S mutants revealed that the AddAB protein complex contains a subane [4Fe–4S] cluster which serves as a molecular staple to stabilize the AddB nuclease domain (102). Moreover, the Fe–S cluster is required for AddAB binding to double-stranded DNA ends and for DNA break processing. AddB mutations in the Fe–S cluster of any one of the four conserved cysteines did not interfere with single-stranded DNA-dependent ATPase or single-stranded DNA translocation catalyzed by the AddAB complex, indicating a preserved architecture of the conserved AddA helicase motifs in the AddAB complex.

A critically important advance for understanding the structural and functional significance of the iron staple domain will be to solve the structure of the AddAB protein–DNA complex. Structural details should help to reveal how AddAB interacts with DNA ends and how the AddB nuclease domain is positioned in relation to the AddA helicase/nuclease domains in the protein–DNA complex. This should yield new insight to the architecture and function of AddAB and perhaps other specialized DNA processing enzymes, such as eukaryotic Dna2 that

also possesses a conserved Fe–S staple domain (102), in DSB strand resection step and other aspects of DNA metabolism.

Dna2

Although mutations in the Fe–S cluster did not affect AddAB ATPase or translocase activity, the presence of an Fe–S domain affected the protein complex's ability to bind broken DNA, a result that is likely to be relevant to the function of the conserved iron staple nuclease domain in other DNA repair proteins, including the SF1 Dna2 helicase–nuclease (Figure 5), a eukaryotic protein implicated in DSB end resection, Okazaki fragment processing and telomere stabilization (103,104). Given that Dna2 is essential for cellular DNA replication, it seems likely that mutations in the Fe–S domain of Dna2 will be poorly tolerated. Although biochemical studies with purified proteins and genetic analysis in yeast suggest that a Dna2-independent EXO1-dependent pathway of DSB end resection exists (105,106), the endonucleoytic function provided by the Dna2 pathway may be important for strand resection of DNA sequences in a BLM helicase-dependent manner (105). It will be incumbent to extract information from biochemical and structural studies of AddAB and apply this insight to the related iron staple nuclease Dna2.

Recent studies have provided evidence that in addition to its nuclear functions, Dna2 can be found in mitochondria and is important for flap processing during BER and the fidelity of DNA replication in mitochondria (107,108). Understanding how Dna2 balances its duties between mitochondria and the nucleus is a high priority. Presumably, the Fe–S domain of Dna2 is required for enzymatic function and its cellular roles; however, no biochemical or genetic studies have directly tested this. A defect in the synthesis of Fe–S clusters is responsible for mitochondrial dysfunction, leading to nuclear genomic instability (109). Defects in Fe–S assembly due to a deficiency in the iron storage/transport protein frataxin also lead to genomic instability and defective BER (110), suggesting that the conserved

Fe–S cluster in Dna2 and other DNA repair/replication proteins may be crippled due to the frataxin deficiency; however, this remains to be shown. Further studies are required to ascertain the importance of the Fe–S staple domain in Dna2 for its nuclear and mitochondrial functions, and the influence of iron homeostasis in this respect.

REDOX ACTIVE Fe–S CLUSTERS: IMPLICATIONS FOR HELICASE MECHANISM

It has been speculated that DNA repair proteins with Fe–S clusters may use their redox properties to scan the genome for DNA damage by sensing DNA charge transport that is mediated by base pair stacking (14). For example, it was proposed that the bacterial redox-sensitive transcription regulator SoxR becomes activated by DNA-mediated charge transport (111). Fe–S cluster BER enzymes (e.g. MutY, Endonuclease III) may exploit DNA charge transport to more efficiently scan and recognize regions of DNA for oxidative base lesions (84). An intriguing aspect of both SoxR and the DNA repair glycosylases is that their redox activities are altered upon DNA binding (84,85), suggesting a possible mechanism for assaying DNA-mediated signaling. This may be relevant to Fe–S cluster helicases and helicase–nucleases, particularly those that play a role in DNA damage recognition or verification.

Recently, it was shown that the ATPase/helicase activity of human XPD was required for the immobilization of the helicase at UV-induced DNA damage foci in living cells (112). *In vitro* studies revealed that purified recombinant archaeal XPD is sequestered at a single cyclobutane pyrimidine dimer when it is positioned in the strand that it translocates with a 5′–3′ polarity; moreover, despite its inability to translocate, XPD ATPase activity is stimulated upon encountering the lesion (112). Understanding if the redox potential of the Fe–S cluster plays a role in XPD trapping at the site of DNA damage would provide insight to the strand- and site-selective DNA lesion demarcation by XPD. Given the importance of lesion verification in the process of NER, a putative role of damage sensing or ATPase-activated helicase sequestration at the DNA lesion via the XPD Fe–S cluster is worth further investigation.

The Barton lab showed that an archaeal XPD has DNA-mediated redox potential similar to that of the Fe–S cluster BER proteins (113). The signal intensity corresponded to the equivalent of a one-electron redox couple of the 4Fe–4S cluster. An important advance was made by the demonstration that SaXPD displayed ATP-dependent electrochemistry, consistent with the idea that the electrochemical signal is coupled to mechanical movement of the helicase as it translocates on DNA. This feature would distinguish the ATP-dependent action of a helicase from a BER Fe–S glycosylase, which functions in an ATP-independent manner. The authors went on to show that the ATP-dependent electrochemistry of SaXPD was maximal when the helicase was placed on a surface that had a 5′ single-stranded DNA tailed duplex immobilized to it. This result was consistent with the known 5′–3′

polarity of XPD, suggesting that its change in redox potential is associated with DNA conformational alterations associated with ATP-dependent duplex unwinding. Overall, the work is highly significant because it raises the strong possibility that Fe–S cluster helicases like XPD represent a specialized class of DNA translocating enzymes that utilize electronic mediated signaling via the DNA molecule to perform its cellular functions in DNA replication, repair and/or transcription. However, a limitation is the lack of understanding of the role of Fe–S cluster redox activity in the regulation of eukaryotic XPD as a component of TFIIH complex during cellular DNA repair or transcription.

The significance of Fe–S clusters in DNA helicases has been a topic of interest (114). In addition to its structural role, the Fe–S cluster may be important for sensing unusual DNA structures whether it is a covalent base adduct or sequence element with an alternate conformation. This theme may apply to Fe–S cluster DNA repair helicases, such as FANCJ that is implicated in ICL repair and G-quadruplex DNA metabolism. In terms of base damage, FANCJ is capable of sensing an oxidative base damage (thymine glycol) in either the translocating or non-translocating strands of duplex DNA (115). This behavior of FANCJ was fairly unique, since the DNA unwinding reactions catalyzed by other DNA helicases tested were inhibited in a strand-specific manner (WRN, BLM, RECQ1) or resistant to inhibition by the thymine glycol positioned in either strand (UvrD, DnaB and the Fe–S domain helicase DinG) (115).

Understanding how the redox activity of the Fe–S cluster plays a role in DNA recognition is an important question. While a specific mechanism has not yet been elucidated, a theoretical study of DNA damage recognition through electron transfer mediated by the 4Fe–4S complex of the DNA glycolsylase MutY that builds upon the studies of the Barton lab suggested a setting in which charge transfer stabilizes a specific conformation of the protein that places it in the recognition mode preferentially over the non-specific binding conformation, providing a means for the DNA repair protein to localize the damaged site in an efficient manner (116). This model proposes that in order for electron transfer mediated by the Fe–S redox activity to be relevant to the process of scanning DNA for sites of damage, the rate of electron transport from donor to acceptor should be greater than the rate of protein diffusion along DNA. Such electron tunneling between DNA repair proteins may be mediated by efficient electron transport through the DNA; however, this theory remains to be proven.

Redox signaling by Fe–S cluster helicases may also play a role in the catalytic reaction mechanism. A hypothesis which remains to be tested is that Fe–S cluster helicase molecules bound to DNA may communicate with each other by changes in redox activity that are mediated through DNA charge transport (Figure 6). The groundwork for this hypothesis was provided by the Raney lab which provided evidence that the Dda helicase monomer, which incidentally lacks an Fe–S cluster, functionally cooperates with other DNA-bound monomers (117). They were able to show that increasing the length of the
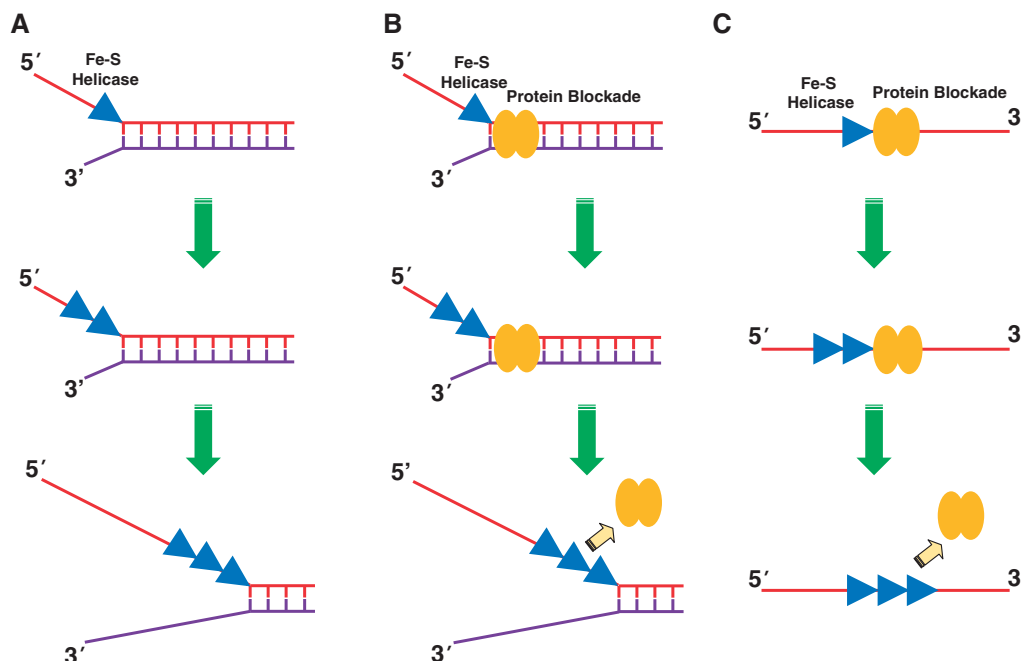
Figure 6. Models depicting functional cooperativity between Fe–S cluster helicase molecules as they unwind DNA duplexes (**A**), or remove proteins bound to double-stranded DNA (**B**) or single-stranded DNA (**C**). Models are adapted by analogy from ones proposed for bacteriophage T4 Dda helicase (118,120).

single-stranded DNA overhang enhanced unwinding of the adjacent duplex by Dda. Their results suggested a model in which multiple Dda molecules bound to the same substrate displayed greater processivity for DNA unwinding. Similarly, multiple NS3 helicase molecules bound to the single-stranded DNA loading region of a partial duplex substrate are required for optimal unwinding (118). More recently, biochemical studies have provided evidence for functional cooperativity between *E. coli* RecQ helicase monomers (119). Although Dda, NS3 and RecQ helicases lack the conserved Fe–S cluster, these proteins may utilize other motifs to communicate between helicase molecules by DNA-mediated redox signaling or another mechanism. Nonetheless, it will be of interest to determine if Fe–S cluster helicase molecules functionally cooperate during DNA unwinding and if the redox function plays a role.

In addition to DNA unwinding, multiple helicase molecules loaded on the same single-stranded DNA molecule may cooperate to facilitate protein displacement (120), a function that may be relevant to the role of certain helicases to facilitate DNA replication or transcription, as mentioned earlier. It has been proposed that the presence of multiple motors may serve to prevent backward displacement on the single-stranded DNA, resulting in an elevated force production to displace protein from the DNA molecule. It is conceivable that Fe–S cluster helicases have emerged as a group of proteins that utilize their ability to alter redox potential in order to communicate between DNA-bound helicase molecules during the task of protein blockade clearance (Figure 6). For example, the ability of certain Fe–S helicases

(e.g. FANCJ, XPD) to efficiently unwind DNA and/or strip proteins off the DNA may be mechanistically facilitated by the Fe–S cluster.

It will be of interest to determine if any of the Fe–S cluster helicases behave in a cooperative manner, and if the redox activity of the Fe–S cluster plays a role in this capacity. In this regard, previously we showed that inhibition of FANCJ helicase activity by a polyglycol linkage that disrupts the sugar phosphate backbone can be overcome by an increased length in the 5′ single-stranded DNA loading tail (70). This suggested a model in which a leading FANCJ helicase molecule is pushed forward by other FANCJ helicase molecules loaded behind it to complete unwinding. Whether or not the redox activity of the FANCJ Fe–S cluster plays a role in the unwinding mechanism remains to be experimentally tested. Single turnover kinetic analyses of DNA helicases such as FANCJ and selected site-directed Fe–S cluster mutants should provide insight to the possibility that the helicase monomers display functional cooperativity in order to efficiently perform their unwinding or protein displacement functions.

Finally, it is quite interesting that all Fe–S cluster helicases characterized to date (FANCJ, XPD, ChlR1, DinG) display a 5′–3′ polarity for DNA unwinding (28). Human RTEL was shown to displace the invading strand of a D-loop substrate provided that the third strand is a 3′ strand invasion with a free 5′ single-stranded DNA, consistent with a predicted 5′–3′ polarity (121). It should be clarified that a Fe–S cluster is not required for 5′–3′ polarity, since a number of 5′–3′ DNA helicases exist which lack a Fe–S cluster. Nonetheless, it is provocative

to consider that the Fe–S cluster may constitute a specialized structural element that helps to dictate the directionality of DNA translocation for the Fe–S domain helicases. The crystal structure of TaXPD bound to single-stranded DNA (Figure 3) (45) suggests that translocation directionality is dictated by conformational changes within the motor domain that determine ATP-driven enzyme translocation in a directional manner. It is also possible that the redox activity imposed by the Fe–S cluster may enable individual FANCJ proteins 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.

There is very little information yet concerning the functional importance of the Fe–S cluster in helicase–nuclease enzymes. One hypothesis is that the conserved Fe–S staple domain in AddAB may regulate the nuclease reaction mechanism by sensing recombination hotspots via alterations in its redox activity at consensus DNA sequence elements. It is anticipated that the endonuclease activity catalyzed by Dna2 necessary for Okazaki fragment processing is tightly regulated and may also involve redox signaling mediated by the Fe–S cluster domain. It will be informative to dissect the structural versus regulatory function of the Fe–S staple domain in the Dna2/AddAB helicase–nucleases.

SUMMARY

Although the evidence is persuasive that the conserved Fe–S cluster domain found in XPD, FANCJ and related DNA helicases plays an important structural and functional role, it is less clear how DNA-mediated redox signaling is important from a mechanistic standpoint. Given the likelihood that DNA charge transport plays an important role in the movement of Fe–S cluster proteins during DNA damage surveillance, it seems probable that Fe–S cluster helicases would exploit the redox active domain for its catalytic functions in ATP-dependent DNA duplex unwinding or protein displacement. This is clearly an important area of investigation as it will lead to new insights to the mechanism of action of Fe–S cluster helicases, which are required for a normal level of DNA repair and maintenance of genomic stability.

ACKNOWLEDGEMENTS

The authors wish to thank Drs Jill Fuss and John Tainer for kindly providing a figure of the apo-SaXPD structure and Drs Jochen Kuper and Caroline Kisker for kindly providing a figure of the TaXPD–DNA complex. We thank these scientists and Dr Malcolm White for helpful discussions.

FUNDING

Conflict of interest statement. None declared.

REFERENCES

1. Py,B. and Barras,F. (2010) Building Fe-S proteins: bacterial strategies. *Nat. Rev. Microbiol.*, **8**, 436–446.
2. Lill,R. (2009) Function and biogenesis of iron-sulphur proteins. *Nature*, **460**, 831–838.
3. Ye,H. and Rouault,T.A. (2010) Erythropoiesis and iron sulfur cluster biogenesis. *Adv Hematol.*, 329–394.
4. Xu,X.M. and Moller,S.G. (2008) Iron-sulfur cluster biogenesis systems and their crosstalk. *Chembiochem.*, **9**, 2355–2362.
5. Lill,R. and Muhlenhoff,U. (2006) Iron-sulfur protein biogenesis in eukaryotes: components and mechanisms. *Annu. Rev. Cell Dev. Biol.*, **22**, 457–486.
6. Sheftel,A., Stehling,O. and Lill,R. (2010) Iron-sulfur proteins in health and disease. *Trends Endocrinol. Metab.*, **21**, 302–314.
7. Rouault,T.A. and Tong,W.H. (2008) Iron-sulfur cluster biogenesis and human disease. *Trends Genet.*, **24**, 398–407.
8. 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.
9. Lu,A.L. and Wright,P.M. (2003) Characterization of an Escherichia coli mutant MutY with a cysteine to alanine mutation at the iron-sulfur cluster domain. *Biochemistry*, **42**, 3742–3750.
10. David,S.S. and Williams,S.D. (1998) Chemistry of glycosylases and endonucleases involved in base-excision repair. *Chem. Rev.*, **98**, 1221–1262.
11. Hinks,J.A., Evans,M.C., De,M.Y., Sartori,A.A., Jiricny,J. and Pearl,L.H. (2002) An iron-sulfur cluster in the family 4 uracil-DNA glycosylases. *J. Biol. Chem.*, **277**, 16936–16940.
12. Lukianova,O.A. and David,S.S. (2005) A role for iron-sulfur clusters in DNA repair. *Curr. Opin. Chem. Biol.*, **9**, 145–151.
13. Romano,C.A., Sontz,P.A. and Barton,J.K. (2011) Mutants of the base excision repair glycosylase, endonuclease III: DNA charge transport as a first step in lesion detection. *Biochemistry*, **50**, 6133–6145.
14. Genereux,J.C., Boal,A.K. and Barton,J.K. (2010) DNA-mediated charge transport in redox sensing and signaling. *J Am. Chem. Soc.*, **132**, 891–905.
15. Fleischmann,C., Peto,J., Cheadle,J., Shah,B., Sampson,J. and Houlston,R.S. (2004) Comprehensive analysis of the contribution of germline MYH variation to early-onset colorectal cancer. *Int. J. Cancer*, **109**, 554–558.
16. Klinge,S., Hirst,J., Maman,J.D., Krude,T. and Pellegrini,L. (2007) An iron-sulfur domain of the eukaryotic primase is essential for RNA primer synthesis. *Nat. Struct. Mol. Biol.*, **14**, 875–877.
17. 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.
18. Vaithiyalingam,S., Warren,E.M., Eichman,B.F. and Chazin,W.J. (2010) Insights into eukaryotic DNA priming from the structure and functional interactions of the 4Fe-4S cluster domain of human DNA primase. *Proc. Natl Acad. Sci. USA*, **107**, 13684–13689.
19. Lohman,T.M., Tomko,E.J. and Wu,C.G. (2008) Non-hexameric DNA helicases and translocases: mechanisms and regulation. *Nat. Rev. Mol. Cell. Biol.*, **9**, 391–401.
20. 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.
21. Brosh,R.M. Jr and Bohr,V.A. (2007) Human premature aging, DNA repair and RecQ helicases. *Nucleic Acids Res.*, **35**, 7527–7544.

22. Patel,S.S. and Donmez,I. (2006) Mechanisms of helicases. *J. Biol. Chem.*, **281**, 18265–18268.

23. Pyle,A.M. (2008) Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu. Rev. Biophys.*, **37**, 317–336.

24. Lohman,T.M. and Bjornson,K.P. (1996) Mechanisms of helicase-catalyzed DNA unwinding. *Annu. Rev. Biochem.*, **65**, 169–214.

25. Singh,D.K., Ghosh,A.K., Croteau,D.L. and Bohr,V.A. (2011) RecQ helicases in DNA double strand break repair and telomere maintenance. *Mutat. Res.*, doi:10.1016/j.mrfmmm.2011.06.002.

26. Dillingham,M.S. (2011) Superfamily I helicases as modular components of DNA-processing machines. *Biochem Soc. Trans.*, **39**, 413–423.

27. Bernstein,K.A., Gangloff,S. and Rothstein,R. (2010) The RecQ DNA helicases in DNA repair. *Annu. Rev. Genet.*, **44**, 393–417.

28. Wu,Y., Suhasini,A.N. and Brosh,R.M. Jr (2009) Welcome the family of FANCJ-like helicases to the block of genome stability maintenance proteins. *Cell Mol. Life Sci.*, **66**, 1209–1222.

29. Lee,J.Y. and Yang,W. (2006) UvrD helicase unwinds DNA one base pair at a time by a two-part power stroke. *Cell.*, **127**, 1349–1360.

30. Velankar,S.S., Soultanas,P., Dillingham,M.S., Subramanya,H.S. and Wigley,D.B. (1999) Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. *Cell.*, **97**, 75–84.

31. Singleton,M.R., Scaife,S. and Wigley,D.B. (2001) Structural analysis of DNA replication fork reversal by RecG. *Cell.*, **107**, 79–89.

32. Yao,N., Hesson,T., Cable,M., Hong,Z., Kwong,A.D., Le,H.V. and Weber,P.C. (1997) Structure of the hepatitis C virus RNA helicase domain. *Nat. Struct.Biol.*, **4**, 463–467.

33. 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.

34. Sengoku,T., Nureki,O., Nakamura,A., Kobayashi,S. and Yokoyama,S. (2006) Structural basis for RNA unwinding by the DEAD-box protein Drosophila Vasa. *Cell.*, **125**, 287–300.

35. Richards,J.D., Johnson,K.A., Liu,H., McRobbie,A.M., McMahon,S., Oke,M., Carter,L., Naismith,J.H. and White,M.F. (2008) Structure of the DNA repair helicase hel308 reveals DNA binding and autoinhibitory domains. *J. Biol. Chem.*, **283**, 5118–5126.

36. 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.

37. Fairman-Williams,M.E., Guenther,U.P. and Jankowsky,E. (2010) SF1 and SF2 helicases: family matters. *Curr. Opin. Struct. Biol.*, **20**, 313–324.

38. 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.

39. Laine,J.P., Mocquet,V. and Egly,J.M. (2006) TFIIH enzymatic activities in transcription and nucleotide excision repair. *Methods Enzymol.*, **408**, 246–263.

40. Egly,J.M. and Coin,F. (2011) A history of TFIIH: two decades of molecular biology on a pivotal transcription/repair factor. *DNA Repair*, **10**, 714–721.

41. 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.

42. 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.

43. 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.

44. 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.

45. Kuper,J., Wolski,S.C., Michels,G. and Kisker,C. (2012) Functional and structural studies of the nucleotide excision repair helicase XPD suggest a polarity for DNA translocation. *EMBO J.*, **31**, 494–502.

46. Pugh,R.A., Wu,C.G. and Spies,M. (2012) Regulation of translocation polarity by helicase domain 1 in SF2B helicases. *EMBO J.*, **31**, 503–514.

47. 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.

48. Botta,E., Nardo,T., Lehmann,A.R., Egly,J.M., Pedrini,A.M. and Stefanini,M. (2002) Reduced level of the repair/transcription factor TFIIH in trichothiodystrophy. *Hum. Mol. Genet.*, **11**, 2919–2928.

49. 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. *et al.* (2001) BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. *Cell*, **105**, 149–160.

50. Seal,S., Thompson,D., Renwick,A., Elliott,A., Kelly,P., Barfoot,R., Chagtai,T., Jayatilake,H., Ahmed,M., Spanova,K. *et al.* (2006) Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat. Genet.*, **38**, 1239–1241.

51. Levitus,M., Waisfisz,Q., Godthelp,B.C., de,V.Y., Hussain,S., Wiegant,W.W., Elghalbzouri-Maghrani,E., Steltenpool,J., Rooimans,M.A., Pals,G. *et al.* (2005) The DNA helicase BRIP1 is defective in Fanconi anemia complementation group J. *Nat. Genet.*, **37**, 934–935.

52. Levran,O., Attwooll,C., Henry,R.T., Milton,K.L., Neveling,K., Rio,P., Batish,S.D., Kalb,R., Velleuer,E., Barral,S. *et al.* (2005) The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat. Genet.*, **37**, 931–933.

53. 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.

54. Walsh,T. and King,M.C. (2007) Ten genes for inherited breast cancer. *Cancer Cell*, **11**, 103–105.

55. Moldovan,G.L. and D'Andrea,A.D. (2009) How the Fanconi anemia pathway guards the genome. *Annu. Rev. Genet.*, **43**, 223–249.

56. Ali,A.M., Singh,T.R. and Meetei,A.R. (2009) FANCM-FAAP24 and FANCJ: FA proteins that metabolize DNA. *Mutat. Res.*, **668**, 20–26.

57. 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. USA*, **101**, 2357–2362.

58. 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.

59. London,T.B., Barber,L.J., Mosedale,G., Kelly,G.P., Balasubramanian,S., Hickson,I.D., Boulton,S.J. and Hiom,K. (2008) FANCJ is a structure-specific DNA helicase associated with the maintenance of genomic G/C tracts. *J. Biol. Chem.*, **283**, 36132–36139.

60. 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.

61. 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.

62. Cannavo,E., Gerrits,B., Marra,G., Schlapbach,R. and Jiricny,J. (2007) Characterization of the interactome of the human MutL homologues MLH1, PMS1, and PMS2. *J. Biol. Chem.*, **282**, 2976–2986.

63. 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.

64. Suhasini,A.N., Rawtani,N.A., Wu,Y., Sommers,J.A., Sharma,S., Mosedale,G., North,P.S., Cantor,S.B., Hickson,I.D. and Brosh,R.M. Jr (2011) Interaction between the helicases genetically linked to Fanconi anemia group J and Bloom's syndrome. *EMBO J.*, **30**, 692–705.

65. 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.

66. Gong,Z., Kim,J.E., Leung,C.C., Glover,J.N. and Chen,J. (2010) BACH1/FANCJ Acts with TopBP1 and Participates Early in DNA Replication Checkpoint Control. *Mol. Cell*, **37**, 438–446.

67. Wu,Y. and Brosh,R.M. Jr (2009) FANCJ helicase operates in the Fanconi Anemia DNA repair pathway and the response to replicational stress. *Curr. Mol. Med.*, **9**, 470–482.

68. Wu,Y., Sommers,J.A., Suhasini,A.N., Leonard,T., Deakyne,J.S., Mazin,A.V., Shin-ya,K., Kitao,H. and Brosh,R.M. Jr (2010) Fanconi anemia group J mutation abolishes its DNA repair function by uncoupling DNA translocation from helicase activity or disruption of protein-DNA complexes. *Blood*, **116**, 3780–3791.

69. Wu,Y. and Brosh,R.M. Jr (2010) Helicase-inactivating mutations as a basis for dominant negative phenotypes. *Cell Cycle*, **9**, 4080–4090.

70. 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.

71. 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.

72. 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.

73. van der Lelij,P., Chrzanowska,K.H., Godthelp,B.C., Rooimans,M.A., Oostra,A.B., Stumm,M., Zdzienicka,M.Z., Joenje,H. and De Winter,J.P. (2010) Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1. *Am. J. Hum. Genet.*, **86**, 262–266.

74. Skibbens,R.V. (2004) Chl1p, a DNA helicase-like protein in budding yeast, functions in sister-chromatid cohesion. *Genetics*, **166**, 33–42.

75. Mayer,M.L., Pot,I., Chang,M., Xu,H., Aneliunas,V., Kwok,T., Newitt,R., Aebersold,R., Boone,C., Brown,G.W. et al. (2004) Identification of protein complexes required for efficient sister chromatid cohesion. *Mol. Biol. Cell.*, **15**, 1736–1745.

76. 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.

77. 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.

78. 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.

79. Chung,G., O'Neil,N.J. and Rose,A.M. (2011) CHL-1 provides an essential function affecting cell proliferation and chromosome stability in Caenorhabditis elegans. *DNA Repair*, **10**, 1174–1182.

80. 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.

81. Wu,Y., Sommers,J.A., Khan,I., de,W.J. and Brosh,R. (2011) Biochemical characterization of Warsaw Breakage Syndrome helicase. *J. Biol. Chem.*, **287**, 1007–1021.

82. Inoue,A., Hyle,J., Lechner,M.S. and Lahti,J.M. (2011) Mammalian ChlR1 has a role in heterochromatin organization. *Exp. Cell Res.*, **317**, 2522–2535.

83. Ren,B., Duan,X. and Ding,H. (2009) Redox control of the DNA damage-inducible protein DinG helicase activity via its iron-sulfur cluster. *J. Biol. Chem.*, **284**, 4829–4835.

84. Boal,A.K., Yavin,E., Lukianova,O.A., O'Shea,V.L., David,S.S. and Barton,J.K. (2005) DNA-bound redox activity of DNA repair glycosylases containing [4Fe-4S] clusters. *Biochemistry*, **44**, 8397–8407.

85. Gorodetsky,A.A., Dietrich,L.E., Lee,P.E., Demple,B., Newman,D.K. and Barton,J.K. (2008) DNA binding shifts the redox potential of the transcription factor SoxR. *Proc. Natl Acad. Sci. USA*, **105**, 3684–3689.

86. Boubakri,H., de Septenville,A.L., Viguera,E. and Michel,B. (2010) The helicases DinG, Rep and UvrD cooperate to promote replication across transcription units in vivo. *EMBO J.*, **29**, 145–157.

87. Sommers,J.A., Rawtani,N., Gupta,R., Bugreev,D.V., Mazin,A.V., Cantor,S.B. and Brosh,R.M. Jr (2009) FANCJ uses its motor ATPase to destabilize protein-DNA complexes, unwind triplexes, and inhibit RAD51 strand exchange. *J. Biol. Chem.*, **284**, 7505–7517.

88. Honda,M., Park,J., Pugh,R.A., Ha,T. and Spies,M. (2009) Single-molecule analysis reveals differential effect of ssDNA-binding proteins on DNA translocation by XPD helicase. *Mol. Cell*, **35**, 694–703.

89. Atkinson,J., Gupta,M.K., Rudolph,C.J., Bell,H., Lloyd,R.G. and McGlynn,P. (2011) Localization of an accessory helicase at the replisome is critical in sustaining efficient genome duplication. *Nucleic Acids Res.*, **39**, 949–957.

90. McGlynn,P. (2011) Helicases that underpin replication of protein-bound DNA in Escherichia coli. *Biochem Soc. Trans.*, **39**, 606–610.

91. 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.

92. 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.

93. Ding,H., Schertzer,M., Wu,X., Gertsenstein,M., Selig,S., Kammori,M., Pourvali,R., Poon,S., Vulto,I., Chavez,E. et al. (2004) Regulation of murine telomere length by Rtel: an essential gene encoding a helicase-like protein. *Cell*, **117**, 873–886.

94. Sfeir,A., Kosiyatrakul,S.T., Hockemeyer,D., MacRae,S.L., Karlseder,J., Schildkraut,C.L. and de,L.T. (2009) Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell*, **138**, 90–103.

95. Barber,L.J., Youds,J.L., Ward,J.D., McIlwraith,M.J., O'Neil,N.J., Petalcorin,M.I., Martin,J.S., Collis,S.J., Cantor,S.B., Auclair,M. et al. (2008) RTEL1 maintains genomic stability by suppressing homologous recombination. *Cell*, **135**, 261–271.

96. McEachern,M.J., Krauskopf,A. and Blackburn,E.H. (2000) Telomeres and their control. *Annu. Rev. Genet.*, **34**, 331–358.

97. Palm,W. and de,L.T. (2008) How shelterin protects mammalian telomeres. *Annu. Rev. Genet.*, **42**, 301–334.

98. Calado,R.T. and Young,N.S. (2009) Telomere diseases. *N. Engl. J Med.*, **361**, 2353–2365.

99. Heiss,N.S., Knight,S.W., Vulliamy,T.J., Klauck,S.M., Wiemann,S., Mason,P.J., Poustka,A. and Dokal,I. (1998) X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat. Genet.*, **19**, 32–38.

100. Mitchell,J.R., Wood,E. and Collins,K. (1999) A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*, **402**, 551–555.

101. 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.

102. Yeeles,J.T., Cammack,R. and Dillingham,M.S. (2009) An iron-sulfur cluster is essential for the binding of broken DNA by AddAB-type helicase-nucleases. *J. Biol. Chem.*, **284**, 7746–7755.

103. Kang,Y.H., Lee,C.H. and Seo,Y.S. (2010) Dna2 on the road to Okazaki fragment processing and genome stability in eukaryotes. *Crit. Rev. Biochem. Mol. Biol.*, **45**, 71–96.

104. Paeschke,K., McDonald,K.R. and Zakian,V.A. (2010) Telomeres: structures in need of unwinding. *FEBS Lett.*, **584**, 3760–3772.

105. Nimonkar,A.V., Genschel,J., Kinoshita,E., Polaczek,P., Campbell,J.L., Wyman,C., Modrich,P. and Kowalczykowski,S.C. (2011) BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev.*, **25**, 350–362.

106. Shim,E.Y., Chung,W.H., Nicolette,M.L., Zhang,Y., Davis,M., Zhu,Z., Paull,T.T., Ira,G. and Lee,S.E. (2010) Saccharomyces cerevisiae Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. *EMBO J.*, **29**, 3370–3380.

107. Zheng,L., Zhou,M., Guo,Z., Lu,H., Qian,L., Dai,H., Qiu,J., Yakubovskaya,E., Bogenhagen,D.F., Demple,B. *et al.* (2008) Human DNA2 is a mitochondrial nuclease/helicase for efficient processing of DNA replication and repair intermediates. *Mol. Cell*, **32**, 325–336.

108. Duxin,J.P., Dao,B., Martinsson,P., Rajala,N., Guittat,L., Campbell,J.L., Spelbrink,J.N. and Stewart,S.A. (2009) Human Dna2 is a nuclear and mitochondrial DNA maintenance protein. *Mol. Cell Biol.*, **29**, 4274–4282.

109. Veatch,J.R., McMurray,M.A., Nelson,Z.W. and Gottschling,D.E. (2009) Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell*, **137**, 1247–1258.

110. Thierbach,R., Drewes,G., Fusser,M., Voigt,A., Kuhlow,D., Blume,U., Schulz,T.J., Reiche,C., Glatt,H., Epe,B. *et al.* (2010) The Friedreich's ataxia protein frataxin modulates DNA base excision repair in prokaryotes and mammals. *Biochem. J.*, **432**, 165–172.

111. Lee,P.E., Demple,B. and Barton,J.K. (2009) DNA-mediated redox signaling for transcriptional activation of SoxR. *Proc. Natl Acad. Sci. USA*, **106**, 13164–13168.

112. Mathieu,N., Kaczmarek,N. and Naegeli,H. (2010) Strand- and site-specific DNA lesion demarcation by the xeroderma pigmentosum group D helicase. *Proc. Natl Acad. Sci. USA*, **107**, 17545–17550.

113. Mui,T.P., Fuss,J.O., Ishida,J.P., Tainer,J.A. and Barton,J.K. (2011) ATP-Stimulated, DNA-Mediated Redox Signaling by XPD, a DNA Repair and Transcription Helicase. *J. Am. Chem. Soc.*, **133**, 16378–16381.

114. White,M.F. (2009) Structure, function and evolution of the XPD family of iron-sulfur-containing 5'->3' DNA helicases. *Biochem. Soc. Trans.*, **37**, 547–551.

115. Suhasini,A.N., Sommers,J.A., Mason,A.C., Voloshin,O.N., Camerini-Otero,R.D., Wold,M.S. and Brosh,R.M. Jr (2009) FANCJ helicase uniquely senses oxidative base damage in either strand of duplex DNA and is stimulated by replication protein A to unwind the damaged DNA substrate in a strand-specific manner. *J. Biol. Chem.*, **284**, 18458–18470.

116. Lin,J.C., Singh,R.R. and Cox,D.L. (2008) Theoretical study of DNA damage recognition via electron transfer from the [4Fe-4S] complex of MutY. *Biophys. J.*, **95**, 3259–3268.

117. Byrd,A.K. and Raney,K.D. (2005) Increasing the length of the single-stranded overhang enhances unwinding of duplex DNA by bacteriophage T4 Dda helicase. *Biochemistry*, **44**, 12990–12997.

118. Tackett,A.J., Chen,Y., Cameron,C.E. and Raney,K.D. (2005) Multiple full-length NS3 molecules are required for optimal unwinding of oligonucleotide DNA in vitro. *J. Biol. Chem.*, **280**, 10797–10806.

119. Li,N., Henry,E., Guiot,E., Rigolet,P., Brochon,J.C., Xi,X.G. and Deprez,E. (2010) Multiple Escherichia coli RecQ helicase monomers cooperate to unwind long DNA substrates: a fluorescence cross-correlation spectroscopy study. *J. Biol. Chem.*, **285**, 6922–6936.

120. Byrd,A.K. and Raney,K.D. (2004) Protein displacement by an assembly of helicase molecules aligned along single-stranded DNA. *Nat. Struct. Mol. Biol.*, **11**, 531–538.

121. Youds,J.L., Mets,D.G., McIlwraith,M.J., Martin,J.S., Ward,J.D., ONeil,N.J., Rose,A.M., West,S.C., Meyer,B.J. and Boulton,S.J. (2010) RTEL-1 enforces meiotic crossover interference and homeostasis. *Science*, **327**, 1254–1258.