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Mechanistic and Biological Considerations of Oxidatively Damaged DNA for Helicase-dependent Pathways of Nucleic Acid Metabolism

Jack D. Crouch and Robert 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

Abstract

Cells are under constant assault from reactive oxygen species that occur endogenously or arise from environmental agents. An important consequence of such stress is the generation of oxidatively damaged DNA, which is represented by a wide range of non-helix distorting and helix-distorting bulkier lesions that potentially affect a number of pathways including replication and transcription; consequently DNA damage tolerance and repair pathways are elicited to help cells cope with the lesions. The cellular consequences and metabolism of oxidatively damaged DNA can be quite complex with a number of DNA metabolic proteins and pathways involved. Many of the responses to oxidative stress involve a specialized class of enzymes known as helicases, the topic of this review. Helicases are molecular motors that convert the energy of nucleoside triphosphate hydrolysis to unwinding of structured polynucleic acids. Helicases by their very nature play fundamentally important roles in DNA metabolism and are implicated in processes that suppress chromosomal instability, genetic disease, cancer, and aging. We will discuss the roles of helicases in response to nuclear and mitochondrial oxidative stress and how this important class of enzymes help cells cope with oxidatively generated DNA damage through their functions in the replication stress response, DNA repair, and transcriptional regulation.

Graphical Abstract

*Corresponding author. Phone: 410-558-8578; Fax: 410-558-8157; broshr@mail.nih.gov.

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Keywords

oxidatively damaged DNA; reactive oxygen species; helicase; replication; DNA repair; DNA metabolism; human disease; nucleic acid

DNA damage induced by reactive oxygen species and its cellular consequences

Reactive oxygen species (ROS) arising from endogenous biochemical processes including mitochondrial oxidative phosphorylation or metabolism (e.g., formaldehyde) or from exogenous sources such as ionizing radiation, environmental pollutants, or chemotherapy drugs can damage proteins, lipids, carbohydrates and nucleic acids [1]. The spectrum of oxidative DNA lesions is quite broad and includes non-helix distorting lesions (e.g., 8-oxo-7,8-dihydroguanine (8-oxoG)) as well as more bulky adducts (interstrand cross-link (ICL) or 5,6-dihydroxy-5,6-dihydrothymine, also called thymine glycol (TG)). Oxidatively induced DNA lesions can occur quite frequently (up to 10,000 apurinic sites or 1,500 8-oxoguanines (8-oxoG) per cell per day) [2] or be quite lethal even at a relatively low frequency (less than 20 ICLs per cell tolerated) [3]. Certain oxidized base lesions may interfere with the normal rate or fidelity of replication, and/or transcription [4]. Misincorporation of a nucleotide across from an oxidized base can become fixed during the next round of replication and result in a mutation. For example, 8-oxoG causes only a very mild perturbation of the DNA double helix [5, 6], but interferes with replication fidelity by causing G:C to T:A substitutions [7–9]. Fortunately, cells are able to at least partially cope with oxidatively damaged DNA via DNA repair pathways [10]. Of the DNA repair pathways, base excision repair (BER) is the most prominent one elicited for the repair of non-bulky oxidative lesions. Nucleotide excision repair (NER) is the choice pathway for bulky adducts, like 5',8-cyclo-2'-deoxyribonucleosides (cPu) lesions [11, 12]. ICL repair, which involves proteins implicated in NER, resolves two DNA strands covalently linked to

one another [13]. There are also mechanisms for cells to tolerate various DNA lesions, including those induced by ROS [1, 2, 4]. DNA damage response triggered by a replication-blocking lesion may generate single-stranded DNA at the fork which initiates a phosphorylation cascade mediated by ataxia telangiectasia and Rad3-related (ATR) kinase that elicits an intra-S phase checkpoint to allow the cell to tolerate or repair the lesion. For those cases in which an oxidative adduct leads to a single-strand break, a member of the poly(ADP)ribose polymerase (PARP) family becomes activated to facilitate signal transduction pathways to help cells repair the damaged DNA and maintain normal cellular homeostasis. In another scenario, a translesion DNA polymerase may catalyze DNA synthesis past a bulky DNA adduct to ensure timely progression of the replication fork. Altogether the cellular consequences and metabolism of oxidative damage and other types of DNA lesions can be quite complex, and a number of DNA metabolic proteins and pathways are involved. Many of the responses to oxidative stress involve a specialized class of enzymes known as helicases, the topic of this review.

Helicases are molecular motors that unwind structured nucleic acids

Helicases are molecular motors that unwind structured polynucleic acids, typically a double helix but also alternatively folded DNA or RNA triplexes and quadruplexes, or DNA-RNA hybrids (e.g., R-loops). Helicases unwind these structures by converting the chemical energy of nucleoside triphosphate hydrolysis to disruption of the many noncovalent hydrogen bonds that stabilize the unimolecular or multi-stranded nucleic acid [14–19]. Another class of molecular motors is comprised of DNA translocases, which contain conserved ATPase motifs similar to those found in DNA helicases but do not act as *bona fide* helicases to generate single-stranded products, but can perform important cellular DNA metabolic functions including chromatin remodeling and replication fork remodeling, as well as DNA branch-migration of recombination intermediates [20–24]. Because DNA contains the genetic information to make RNA and proteins, its integrity is fundamentally important for life. Therefore, helicases and DNA translocases that act upon structured DNA molecules play essential roles in cellular replication, DNA repair, recombination, and transcription. Damage that occurs to DNA by endogenous biochemical processes or environmental agents interfere with these processes, but also can give rise to deleterious mutations that alter coding information. Of particular interest in this review is the role of DNA helicases in the repair of oxidatively generated DNA damage which can arise endogenously or be exogenously induced. We will begin this review by summarizing the effects of site- and strand-specific oxidized base lesions on unwinding by human DNA helicases. Following this, we will discuss the important roles of helicases in response to nuclear oxidative stress. Included in this discussion will be some concepts and hypotheses regarding how certain helicases might facilitate the recognition of oxidatively damaged DNA within specialized sequences or regions of the genome to facilitate DNA metabolic processes such as DNA repair. We devote a special section to DNA helicases which operate in pathways that respond to oxidative stress in the unique environment of mitochondria that are rich in ROS. The biological and clinical outcomes of helicase dysfunction emphasize the importance of this class of enzymes for genomic stability, and suppression of aging, human genetic diseases, and cancer that are often characterized by an aberrant response to oxidative stress [25].

Single oxidative base lesions differentially affect unwinding by human DNA helicases

In many instances helicases or DNA translocases are likely to be among the first proteins to encounter genomic DNA adducts by virtue of their ability to move along single-stranded or double-stranded DNA in a manner dependent on nucleoside triphosphate hydrolysis. Moreover, it has been postulated that certain helicases (e.g., XPB, XPD) play a role in the recognition or verification of bulky DNA damage [26–31]. It is less certain but plausible that other helicases (e.g., RecQ members) may sense DNA damage as a component of the replication stress response [32]. Therefore, it has been of interest to characterize the effects of DNA adducts on helicase-catalyzed DNA unwinding. Biochemical studies have suggested a general rule that bulky lesions inhibit helicases when they are positioned in the strand that the helicase predominantly interacts with and translocates upon (i.e., translocating strand); however, this may be an over-simplification of the phenomena and that there are lesion-specific and helicase-specific effects that reflect the nature of the DNA structural distortion by the adduct and/or the helicase under investigation [33, 34]. This general principle may come into play for less helix-distorting damage such as certain oxidative base lesions as well.

Table 1 lists the effects of two distinct replication-blocking oxidative base lesions on unwinding by human DNA helicases. TG is a helix-distorting lesion that causes the damaged base to assume an extrahelical position [35]; therefore, it poses a formidable block to DNA synthesis past the lesion [36]. The cPu lesion is caused by a second covalent bond between the base and corresponding sugar, which perturbs normal helix twist and base stacking [37, 38]. The cPu adduct interferes with replication [39] and transcription [40]. As shown in Table 1, a single oxidized base lesion nested within one or the other strand of the duplex region of a forked DNA substrate may exert differential effects on helicase-catalyzed duplex DNA unwinding ranging from potent inhibition, modest (2-fold) inhibition, or no detectable inhibition, depending on the human DNA helicase under investigation [41–43]. This is exemplified by the translocating strand TG lesion which potently inhibits FANCI or BLM activity but exerts only a modest effect on WRN or Twinkle activity [42, 43]. FANCI-catalyzed unwinding is strongly sensitive to the TG irrespective of the strand it resides, whereas DNA unwinding by the other human recombinant helicases tested (RECQL1, BLM, Twinkle) are only affected when the lesion is present in the translocating strand. It will be of interest to determine the structural and mechanistic basis for this difference, and in particular why FANCI is so sensitive to the TG even when it is positioned in the non-translocating strand. FANCI contains a conserved iron-sulfur (Fe-S) cluster domain [44, 45], and by analogy this domain also found in thermophilic XPD is presumed to be directly involved in duplex unwinding by serving as a wedge to separate the complementary strands [46, 47]. While it seems likely that the human Fe-S DNA helicases (XPD, FANCI, DDX11, RTEL-1) unwind DNA by a similar mechanism, there may be distinctions as suggested by the observation that DNA unwinding by the bacterial Fe-S helicase DinG was relatively insensitive to the TG, regardless if the adduct resided in either the non-translocating or translocating strand [43]. From a mechanistic standpoint, it would be informative to know if FANCI reaches out ahead of the duplex region to sense the distortion in the double helix

induced by the thymine glycol and if this is responsible for the inhibition of unwinding or if the helicase translocates all the way to the lesion within a couple nucleotides and then becomes blocked from advancement.

Interestingly, inhibition of FANCDJ by the non-translocating strand TG lesion can be overcome by the presence of the human single-stranded DNA binding protein Replication Protein A (RPA) in the reaction mixture [43], which physically interacts with FANCDJ [48] and strongly binds to single-stranded DNA harboring a TG [43]. A model was presented in which FANCDJ partially unwinds a DNA substrate with a TG in the non-translocating strand; the single-stranded DNA with the exposed lesion is tightly bound by RPA, preventing it from reannealing. The remaining duplex is subsequently unwound to completion by RPA. RPA also stimulated RECQ1 helicase activity in a similar strand-specific manner such that RECQ1-catalyzed DNA unwinding was also up-regulated by RPA when the TG was positioned in the non-translocating strand [43]. Therefore, we propose that the described model may be applicable to other RPA-interacting DNA helicases, regardless of their directionality for unwinding.

Human RecQ helicases and their roles in response to nuclear DNA damage induced by oxidative stress

A prominent class of clinically relevant DNA helicases is represented by the RecQ family, named after a bacterial DNA helicase in which the five human DNA helicases (WRN, BLM, RECQL1, RECQL4, RECQL5) share significant sequence homology within the conserved ATPase/helicase motifs [25, 49–51]. Mutations in three of the five human RecQ helicases (WRN, BLM, RECQL4) are linked to diseases characterized by chromosomal instability, defects in the DNA damage response, and clinical features that resemble accelerated aging. Deficiency in the human or mouse RecQ helicase RECQL1/RECQL results in cellular genomic instability [52, 53], and RECQL1 is implicated in cancer suppression [54–56] (see below). *Recql5*-deficient mice are predisposed to cancer, half being lymphomas and the rest being solid tumours of different tissue origins [57]. Therefore, understanding the precise molecular and cellular functions of these DNA helicases in the DNA damage and replication stress response is of great importance. Summarized below are findings from a number of labs which indicate that WRN, RECQL1, and RECQL5 all play roles in the response to nuclear DNA damage caused by oxidative stress. RECQL4 is discussed in a section that appears later in the review dedicated to DNA helicases and the metabolism of mitochondrial oxidatively damaged DNA.

WRN

Cells from Werner syndrome (WS) patients are sensitive to H₂O₂ [58] and depletion of WRN causes accumulation of DNA damage after acute oxidative stress [59]. WRN-depleted cells are hypersensitive to methylmethane sulfonate, an alkylating agent that generates damage primarily repaired by BER [60, 61]. Whole cell extracts from WRN-depleted cells displayed reduced long patch BER [60]. Consistent with these findings, WRN protein physically interacts with and stimulates the catalytic functions of a number of proteins implicated in BER including NEIL1 glycosylase [62], polymerase β [63], and Flap

Endonuclease I (FEN-1) [64, 65]. Therefore, positive cooperativity of WRN exists with players which operate at three critical steps of the BER pathway, namely DNA cleavage of the glycosidic bond between the damaged base and sugar, DNA repair patch synthesis, and flap processing. WRN also interacts physically and functionally with PARP1, a DNA damage sensor and chromatin modifier implicated in BER, to help cells respond to oxidative stress and alkylating agents [66, 67]. Taken together, the experimental data support a model in which WRN is an important player in the oxidative stress response, largely mediated by its regulatory role in BER.

In addition to its role in BER of general genome oxidatively damaged DNA, WRN has been implicated in telomeric DNA stability from studies with mouse models and human cells. Late-generation telomerase and WRN-deficient mice (*Terc*^{-/-} *Wrm*^{-/-}) display aberrant telomeres, genomic instability, clinical features of premature ageing, and cancers typical of Werner syndrome [68–70]. WRN-deficient human cells were observed to be defective in replication of the telomeric guanine (G)-rich lagging-strand template [71, 72]. WRN was found to be localized to telomeres and shown to interact with several proteins of the shelterin complex that protect telomere integrity and modulate WRN's helicase and exonuclease activities on telomeric D-loop structures (T-loops) [73]. Because telomeric DNA repeats are rich in guanine residues and guanines are preferentially attacked by ROS [74, 75], it seems probable that a component of WRN's multi-tasking functions at chromosome ends involves the BER pathway. However, it should be pointed out that to our knowledge no formal role of WRN in the repair of oxidatively damaged DNA at telomeres has yet been reported. This area of research deserves attention, as a number of studies indicate that defective repair of oxidative base damage at telomeres is associated with multiple telomere abnormalities [76–79] and interference of shelterin proteins with telomeric DNA [80]. WRN's ability to resolve G-quadruplex (G4) DNA structures [81, 82] likely to form within telomeres (and other G-rich regions of the genome) may also play a role in the stability of chromosome ends or other specialized genomic DNA regions. See below an elaboration on the topic of helicase involvement in the metabolism of oxidative base damage in duplex DNA or at G-rich sites predicted to form G4.

RECQL5

RECQL5-deleted human cells are sensitive to the oxidative agents menadione or H₂O₂ and endogenously accumulate the oxidized base lesion 8-oxoG as well as strand breaks [83]. Alkaline comet assays showed that RECQL5 loss in human cells elevated the level of formamido pyrimidine DNA glycosylase sensitive sites, indicative of oxidized guanine bases or abasic sites [83]. The idea that RECQL5 may participate directly in the repair of endogenous DNA damage is supported by the observation that the RECQL5 β isoform interacts with and stimulates structure-specific cleavage of 5' flap DNA substrates by FEN-1, a nuclease that is implicated in BER [84]. However, further studies are required to ascertain if RECQL5 suppresses the accumulation of oxidatively damaged DNA by directly participating in the BER pathway. RECQL5's positive regulation of PARP1 and XRCC1 expression may also contribute to BER capacity [83].

RECQL1

Although RECQL1 was the first human RecQ helicase discovered and the most abundant of the five human RecQ helicases, it remains one of the less characterized. Given RECQL1's prominence as a breast cancer susceptibility gene [54–56], both basic science and translational efforts on the helicase have become increasingly important. Work from our lab demonstrated that embryonic fibroblasts from RECQL1-deficient mice [52] or human cells depleted of RECQL1 by RNA interference [53] were sensitive to ionizing radiation, a form of energy that causes oxidation of bases in DNA as well as strand breaks. However, until only recently it was highly speculative if RECQL1 played a role in the response to oxidative stress. The Sharma lab found that human RECQL1-depleted cells were sensitive to H₂O₂; moreover, RECQL1 was found to directly interact with the DNA strand break sensor PARP-1, and acutely recruited to chromatin in cells exposed to H₂O₂ [85]. It is yet undetermined if RECQL1 contributes in some way to the repair of oxidative base damage, or it may serve a less direct role in DNA damage signaling or regulation of the response to oxidative stress. RECQL1 is implicated in the regulation of replication restart after cellular exposure to the topoisomerase inhibitor camptothecin [86], and was found to govern RPA's availability to maintain normal replication dynamics and preserve genomic stability by suppressing the accumulation of DNA damage [87]. An analogous role of RECQL1 to govern RPA's role in response to oxidative stress remains to be seen.

In other work from the Sharma lab, they assessed genome-wide changes in gene expression upon RECQL1 depletion [88]. Their results suggested that even in unchallenged cancer cells, RECQL1 serves to promote expression of genes that play important roles in cell migration, invasion and metastasis. These results are in line with a previous observation that RECQL1 expression is up-regulated in cancer cell lines compared to normal cells [89], and elevated in certain types of cancers [90–92]. Genes down-regulated upon RECQL1 silencing displayed an enrichment of predicted G4-forming sequences in their promoter elements [88]. In addition, RECQL1 preferentially bound G4 motifs in promoters of genes that were down-regulated upon RECQL1 depletion, as demonstrated by Chromatin immunoprecipitation. It is yet unclear the significance of RECQL1's interaction with promoter-associated G4 DNA as RECQL1 is poorly active as a helicase on G-quadruplex DNA substrates [93, 94]. In the future, it will be important to assess RECQL1's role in modulating gene expression in cancer cells exposed to agents that induce DNA damage, particularly oxidative stress given the observations that RECQL1 deficiency sensitizes cells to ionizing radiation or H₂O₂. Co-regulation of gene expression by RECQL1 and the sequence-related WRN and BLM helicases suggests that the potentially overlapping roles of human RecQ helicases in gene expression [95] may contribute to the complexity of the DNA damage response.

Oxidative stress induced effects on DNA charge transport and involvement of Fe-S cluster helicases to locate DNA damage

A family of DNA helicases that possess a conserved iron-sulfur (Fe-S) domain within the helicase core domain play important roles in DNA repair and maintenance of genomic stability [44, 96]. The four human members of the so-called Fe-S cluster helicase family are XPD, FANCI, DDX11 (also designated ChIR1), and RTEL-1. Mutations in XPD are

genetically linked to Xeroderma pigmentosum, and the helicase plays a key role in the DNA damage verification step of the NER pathway [97]. Mutations in FANCD1 are linked to the progressive bone marrow failure disorder Fanconi Anemia (FA) [98–100] and predispose individuals to breast or ovarian cancer (for review, see [101]). DDX11 mutations are linked to Warsaw Breakage syndrome, in which the cells are characterized by perturbed sister chromatid cohesion and the patients have microcephaly, developmental problems, and abnormal skin pigmentation [102, 103]. RTEL-1 mutations are linked to a severe form of Dyskeratosis congenita, known as Hoyeraal-Hreidarsson, characterized by bone marrow failure, predisposition to cancer, and telomere defects [104–106].

A story of emerging interest has developed in which DNA repair proteins like helicases with redox-active Fe-S clusters exploit disruption of DNA charge transport to scan the genome searching for sites of DNA damage (for review, see [107]). This is a fascinating area because even with the increased affinity of certain DNA damage recognition proteins for a DNA adduct and elaborate DNA repair mechanisms, it seems probable that a given lesion located within a sea of millions of base pairs in the human genome would be highly difficult to locate. A potential mechanism to proximally localize BER proteins to oxidized DNA lesions was evidenced by the Barton lab in which they showed that the oxidation state of Fe-S cluster DNA repair glycosylases (*E. coli* EndoIII and MutY) are able to cooperatively sense the disruption of DNA charge transport by a single mismatch to localize to the DNA aberration [108]. Given that Fe-S clusters are found in number of DNA metabolic proteins including glycosylases, primases, helicases, nucleases, transcription factors, RNA polymerases, and RNA methyltransferases [109], it seems probable that such enzymes might exploit their redox potential as they operate in DNA transactions. The Fe-S cluster XPD DNA helicase of *Sulfolobus acidocaldarius* (Sa) was found to display ATP-dependent electrochemistry which may be important its DNA translocation process [110]. In subsequent work, the Barton lab demonstrated that SaXPD collaborates with *E. coli* EndoIII to efficiently redistribute to a duplex DNA mismatch provided that they were both charge transfer competent. This result suggested a mechanism in which the proteins partner to transfer electrons with each other in a manner that is dependent on DNA-mediated charge transfer signaling, and this signaling allows efficient DNA damage scanning and detection [111]. In addition to SaXPD, the DNA helicases *E. coli* DinG [112] and human RTEL-1 [113] possess redox-active Fe-S clusters, suggesting a broad role of Fe-S cluster DNA helicases to facilitate DNA repair processes by scanning for DNA damage. It will be important to ascertain if DNA metabolic proteins such as helicases with redox active Fe-S clusters transmit the signaling information provided by DNA charge transport to facilitate detection of biologically relevant DNA damage such as oxidized bases to aid DNA glycosylases responsible for initiation of BER. In a more general sense, certain base lesions that disrupt electron flow within the DNA double helix may be recognized by Fe-S cluster DNA metabolic proteins via a redox mechanism (Fig. 1).

Oxidative stress, G4-forming sequences, and potential involvement of DNA helicases

Compared to the other three nucleobases (adenine, cytosine, or thymine), guanine residues in DNA are preferentially oxidized by single-electron oxidants [74, 75]; therefore, oxidative damage in G-rich sequence elements (e.g., telomere repeats, ribosomal DNA, promoter elements) [114–116] may be abundant. Thus, it is critical to ascertain how important is the role of DNA helicases to replicate, repair, or facilitate transcription of DNA sequences with oxidized guanines in the nuclear or mitochondrial genomes (Fig. 2). Indeed, there has been much recent interest in the influence of local sequence context and higher order structure of DNA (e.g., G-quadruplexes) on its reactivity to oxidative stress or even repair of oxidative base damage [117–121]. In support of the potential deleterious effects of oxidized G-quadruplex sequences, Zhou et al. showed that DNA glycosylases are unable to initiate repair of 8-oxoG lesions residing within G4 DNA [122]. Thus, a helicase may be required to resolve the structure so repair can occur. Certain Fe-S cluster helicases (FANCF [94, 123], DDX11 [124], RTEL-1 [125]) or RecQ helicases (WRN [81, 82], BLM [126]) able to resolve G4 DNA may coordinate this function with the response to oxidative stress and repair of oxidatively damaged DNA. This may be particularly relevant for the Fe-S cluster helicases like FANCF that efficiently resolves a wide spectrum of G4 DNA substrates [127] because they are potentially capable of sensing DNA damage by their redox activity as described in the previous section. Delaney and Barton examined charge transport from a DNA duplex to an adjacent G-quadruplex and found that the oxidizing radicals are trapped in the G4 [128], lending support to the idea that oxidatively damaged DNA may occur preferentially in these structures presumably abundant in guanine-rich sequences (e.g., telomeres) or mtDNA (discussed later in this review). Moreover, similar to oxidized base lesions, G-quadruplexes themselves may be a signaling molecule to recruit redox-active Fe-S cluster proteins.

Very recently, the Raney lab showed that G4 DNA accumulates in the cytoplasm of human cells exposed to the DNA oxidizing agent H_2O_2 and participates in the assembly of stress granules [129]. This discovery raises the question if a deficiency in a nuclear or cytosolic G4 resolving helicase or G4 binding protein might modulate the oxidative stress response via the newly discovered G4-induced stress granule formation pathway. Indeed, Byrd et al. identified the most abundant human G4-resolving helicase DHX36/RHAU/G4R1 helicase [130] using G4 DNA as bait in the pull-down assay using human cell lysates [129]. Further studies in this exciting area are warranted given the interest in G4 nucleic acids as a potential molecular target in cancer therapies [131, 132].

Fanconi Anemia pathway, oxidative stress, FANCF helicase, and FANCD1 DNA translocase

FA is a hereditary disorder characterized by progressive bone marrow failure, congenital abnormalities, and a predisposition to cancer. Mutations in all 19 genes linked to FA result in cellular hypersensitivity to DNA ICL agents, and the corresponding gene products coordinate with one another and other proteins to remove the DNA ICL and replace it with

undamaged DNA sequence [133]. Thus, FA is recognized as a DNA repair disorder. However, cells from FA individuals are hypersensitive to agents that induce oxidative stress [134] and display elevated oxidatively damaged DNA [135], including 8-oxoG [136], consistent with elevated ROS and mitochondrial dysfunction [137, 138]. The elevated ROS in FA is thought to arise from increased circulatory inflammatory cytokines [139, 140], which in turn may be stimulated by oxidatively damaged DNA (reviewed in [141]) (Fig. 3).

The hematopoietic stem cell attrition characteristic of FA is believed to arise in large part from DNA damage induced by endogenous formaldehyde [142–144]. Defective repair of DNA damage (such as ICLs) caused by endogenous formaldehyde is believed to be responsible for the clinical features of premature ageing associated with FA, including progressive bone marrow failure, organ damage, and cancer. Aside from the role of FA proteins in ICL repair, some FA proteins influence ROS-producing redox reactions [145–147], mitochondrial function [148], and epigenetic modifications [149] which may also contribute to the cellular and clinical features of FA.

FANCI

Of the currently identified 19 FA gene products, FANCI is the only *bona fide* DNA helicase in the pathway. FANCI unwinds conventional forked duplex DNA substrates [150] and is inhibited by a single TG but tolerates 8-oxoG [43], as discussed above. There is much interest in the role of FANCI and other DNA helicases to deal with replication stress [151]. FANCI-deficient cells are sensitive to the replication inhibitors hydroxyurea and aphidicolin [152–154], but have not been reported to be affected by oxidizing agents. FANCI and the Bloom's syndrome helicase (BSH) co-localize after replication stress and the two helicases partner to unwind damaged DNA with a sugar phosphate backbone discontinuity [154]. Whether or not these two helicases collaborate to unwind damaged DNA containing oxidized base lesions induced by formaldehyde that impede the replication fork remains to be seen. FANCI plays a role in the intra-S phase checkpoint through its interaction with TopBP1, allowing ATR activation of checkpoint kinase 1 (Chk1) [155]; however, it is yet unclear if this is relevant to certain forms of oxidative stress. FANCI also resolves a variety of G-quadruplex DNA substrates, including those derived from human telomeric DNA sequence [94] and unimolecular G4 DNA [156]. FANCI can be found at telomeres in telomerase-negative human cells that maintain their chromosome ends via recombination-based alternative lengthening of telomeres (ALT) pathway [157]; however, there is no evidence yet that FANCI plays a physiologically relevant role in telomere metabolism. Nonetheless, FANCI's ability to resolve G4 DNA may be relevant to the metabolism of oxidatively damaged DNA that preferentially occurs in guanine-rich sequences. It remains to be seen if DNA charge transport plays a role in FANCI's interaction with and metabolism of oxidatively damaged DNA.

FANCD1

Like FANCI-deficient cells, FANCD1-deficient cells are hypersensitive to DNA cross-linking agents [158–160]. Human FANCD1 is a DNA translocase capable of disrupting DNA triplex structures, but does not perform the classic unwinding of duplex DNA molecules [159]. However, FANCD1 is able to catalyze DNA fork regression and branch-migration as well as

dissociate three-stranded D-loop DNA structures that are intermediates of homologous recombination [161, 162]. To our knowledge, the effects of oxidized DNA lesions on these ATP-dependent biochemical activities catalyzed by FANCM have not been assessed. FANCM's catalytic function is implicated in fork regression or replicative traversal of DNA ICLs [163] that are believed to be induced endogenously by oxidative stress or exogenously by certain compounds and chemotherapy drugs (for review, see [13]). Clearly, the precise biochemical pathways whereby endogenous cross-links are created require more investigation. Although there are no reports to our knowledge that FANCM deficiency in human cells confers sensitivity to agents that induce oxidative non-bulky base damage typically repaired by BER, a deficiency in the yeast FANCM ortholog causes sensitivity to the alkylating agent methylmethane sulfonate which introduces DNA base lesions corrected by BER [164, 165].

DNA helicases and the metabolism of mitochondrial oxidatively damaged DNA

In order to produce ATP, mitochondria generate oxygen radicals at a high rate as byproducts of oxidative phosphorylation and the activity of the electron transport chain [166]. Therefore, the elevated ROS cause a significant level of damage to macromolecules in the mitochondrial matrix, including a variety of oxidized base lesions and single strand breaks [167]. Not surprising, BER is a robust DNA repair process in mitochondria which is capable of repairing deaminated, oxidized, and alkylated bases [168]. As listed in Table 2 and discussed in more length below, a number of human DNA helicases localize to mitochondria and serve to modulate BER and other less well characterized responses to oxidative stress and mitochondrial DNA (mtDNA) damage.

XPD

Although XPD helicase, a core component of the general transcription factor TFIIH, is mostly known for its fundamentally important roles in transcription and NER of bulky nuclear DNA damage [97, 169], recent findings indicate that XPD has an additional role in mitochondria. Liu et al. determined that XPD not only resides in human mitochondria, but also that a deficiency in XPD causes increased mitochondrial reactive oxygen species (ROS) and hypersensitivity to H₂O₂ as displayed by an accumulation of a prominent mtDNA 4977-bp deletion (referred to as CD) and elevated oxidatively damaged DNA [170]. The recent demonstration that XPD helicase activity is required for NER of nuclear UV-induced DNA damage [171] prompted Liu et al. [170] to determine if expression of the XPD ATPase-dead mutant XPD-K48R was incapable of restoring repair of oxidatively damaged mtDNA in XPD-depleted cells. Indeed this was found to be the case, suggesting a universally important role of XPD helicase activity in repair of either bulky nuclear DNA adducts or mitochondrial oxidized base lesions. XPD was found to be associated with mitochondrial Tu translation elongation factor (TUFM), and TUFM also plays a role in repair of oxidatively damaged mtDNA [170]; however, if a coordination of XPD with TUFM exists that is important for mtDNA repair remains to be seen. Moreover, it is yet unclear if XPD directly participates with the base excision repair (BER) machinery to correct damaged base lesions which arise in human mtDNA. Lastly, there has been some speculation if loss of nuclear XPD might

affect mitochondria and repair of its genome indirectly because XPD plays an important role in nuclear transcription as well as nuclear DNA repair [97]. Clearly, further studies are warranted to elucidate the direct or indirect functions of XPD and other DNA damage response proteins in mtDNA metabolism and mitochondria's resistance to oxidative stress.

RECQL4

In 2012, three papers appeared in the literature which demonstrated independently that the human RecQ DNA helicase, RECQL4, that is genetically implicated in three distinct hereditary diseases (Rothmund-Thomson syndrome (RTS), Baller Gerold syndrome (BGS) and RAPADILINO) can be found in mitochondria of human cells. Croteau et al. demonstrated that depletion of RECQL4 caused lower reserve capacity (i.e., spare potential energy) compared to control cells indicating a role of RECQL4 in mitochondrial bioenergetics [172]. They also found that the RECQL4-deficient cells displayed elevated endogenous mtDNA damage, as indicated by quantitative PCR [172]. De et al. [173] determined that RECQL4 regulates recruitment of the tumor suppressor molecule p53 to sites of *de novo* mtDNA replication in a manner that is independent of exogenous stress. Finally, Chi et al. [174] reported that RECQL4 depletion caused a marked decrease in mtDNA copy number and increased mitochondrial superoxide production. RECQL4 deficiency also resulted in a reduced capacity for repair of oxidatively damaged mtDNA in cells challenged with menadione, a chemical that causes oxidative stress. The finding of De et al. [173] that RECQL4 is required for optimal *de novo* mtDNA replication is compelling; however, a direct role of RECQL4 in mtDNA replication is still lacking. Further studies with a set of reconstituted mitochondrial replisome proteins and auxiliary factors may be helpful in assigning a functional role of RECQL4 in the replication of the organelle's genome. The fact that RECQL4 is the only known of the five human RecQ helicases to localize to mitochondria is provocative, and perhaps provides a window to see how at least one member of the disease-relevant helicase family has a unique role in nucleic acid metabolism that may be related to a specialized function to deal with oxidative stress rampant in mitochondria.

In work subsequent to the original discoveries that RECQL4 can be found in mitochondria, Gupta et al. [175] showed that RECQL4 and p53 interact with mtDNA polymerase subunits Pol γ A and Pol γ B and regulate their binding to the mtDNA control region, the D-loop. Moreover, RECQL4 independent of its helicase activity was observed to enhance DNA synthesis catalyzed by Pol γ A/Pol γ B *in vitro*. While a story emerges that RECQL4 (and p53) helps to maintain stability of the mitochondrial genome by regulating Poly activity, it is plausible that RECQL4 may also play a role in mtDNA repair. The clinical significance of RECQL4 in mitochondrial dysfunction may manifest itself at multiple levels. For example, a RECQL4 cancer-inducing mutation resulting in deletion of Ala420-Ala463 disrupts RECQL4's interaction with a mitochondrial protein, p32, known to regulate protein localization to the mitochondria. This induces a super-enrichment of RECQL4 in mitochondria and abnormally elevated mtDNA synthesis [176]. Very recent studies from the Sengupta lab provide evidence that when RECQL4 fails to properly localize to mitochondria of human cells, mitochondrial integrity is negatively affected along with a decrease in ATP synthase activity, reduction in membrane potential, and elevated ROS due to superoxide dismutase (SOD) inactivation by a SIRT3-dependent mechanism [177]. As a consequence of

mitochondrial dysfunction due to RECQL4 absence, aerobic glycolysis-dependent cell invasion is stimulated. This led the authors to propose that as an accessory mtDNA replicative helicase, RECQL4 helps to suppress cell invasion during neoplastic transformation.

DNA2

Dna2 was discovered as an important yeast helicase-nuclease required for processing of DNA intermediates during replication and repair of the eukaryote's genome [178, 179]. Exciting advances in 2008 and 2009 from the Shen [180] and Stewart [181] labs and their collaborators revealed that this critical nuclear DNA processing nuclease implicated in lagging strand synthesis could also be found in mitochondria of human cells. A key take-home message from the Zheng et al. [180] study was that DNA2 is required for efficient removal of RNA primers that are used in semi-conservative DNA replication and also 5' flaps that arise during long patch BER. In both processes, the removal of residual ribonucleotides or flap structures is likely to be imperative for mtDNA stability as is the case for nuclear genomic DNA stability. The paper by Duxin et al. [181] was highly significant because it showed that DNA2 depletion not only dismantled mtDNA repair of H₂O₂-induced DNA lesions but also impaired the fidelity of mtDNA replication. Given the still controversial models for mtDNA replication, insights from studies of DNA2 and other proteins implicated in primer removal should be insightful for understanding mechanism of mitochondrial disease pathogenesis (for review on this topic, see [182]).

PIF1

One of the first studies that implicated eukaryotic PIF1 in the response to mitochondrial oxidatively damaged DNA was actually in the model genetic organism *Saccharomyces cerevisiae*. The Doetsch lab took advantage of yeast genetics to create and characterize single, double and triple mutant strains that would enable them to examine how conditions of chronic oxidative stress (*sod2*) and a BER deficiency (*ntg1*) might be affected by loss of PIF1 (*pif1*) [183]. *ntg1 pif1 sod2* strains displayed a profound loss of mtDNA. The *ntg1 pif1* strain was highly sensitive to oxidative stress imposed by co-treatment with antimycin and H₂O₂. Further studies in this work confirmed that Pif1 is an important player to retain respiration competency, indicative of mtDNA stability.

Beyond the response to oxidative stress, yeast PIF1 is known to have essential roles at chromosome ends (telomeres), ribosomal DNA maintenance, and Okazaki fragment processing (for review, see [184–186]). With advances pointing toward an important role of Pif1 in nuclear as well as mitochondrial DNA metabolism in yeast, Futami et al. first reported the localization of human Pif1 in mitochondria as well as nuclei [187]. Surprisingly, we found no publications in the literature that have addressed if human Pif1 plays a direct role in oxidatively damaged DNA repair. This area seems to be greatly understudied, and we anxiously await progress to learn if Pif1 modulates 5' flap processing during BER in the mitochondria where oxidative stress is prominent. This seems a reasonable possibility, as a number of studies indicate a role of Pif1 in Okazaki fragment processing and telomere maintenance by virtue of its ability to efficiently unwind 5' flap DNA structures, a key intermediate of long patch BER, as mentioned above (for review, see [184–186]). Given that

predicted G4 DNA structures believed to impede DNA synthesis are abundant in eukaryotic nuclear and mitochondrial genomes [188–192], it seems probable that PIF1 plays an important role to resolve such mtDNA structures in human cells, as evidence already implicates the homolog in nuclear DNA replication through G4 sequences in *S. cerevisiae* [193] and *S. pombe* [9].

SUV3

SUV3 helicase has been implicated in nuclear messenger RNA turnover and processing in yeast [194, 195]. Minczuk et al. first determined that the human SUV3 orthologue can be localized to mitochondria [196]. Indeed, SUV3 was found to be important for maintaining normal mtDNA copy number and mitochondrial morphology in human cells [197], and play a role in human mitochondrial RNA turnover [198]. Human SUV3 was shown to be involved in a protein complex that modulates messenger RNA polyadenylation in response to cellular energy changes [199]. Although no direct role of SUV3 in oxidatively damaged DNA processing pathways is known, nuclear SUV3 helicase was shown to interact with FEN-1 [200], a structure-specific nuclease critical for BER of oxidized DNA lesions that can also be found in mitochondria of human cells [201]. Nonetheless, a direct role of SUV3 in mitochondrial DNA repair is not apparent at this time.

TWINKLE

The *c10orf2* gene encodes a DNA helicase known as Twinkle, that is an essential component of the mitochondrial replisome [202]. The Twinkle helicase operates at the mtDNA replication fork with the mitochondrial single-stranded binding protein (mtSSB) to unwind the parental duplex DNA circle allowing DNA synthesis by POL γ to proceed smoothly [203]. Recently, Stojkovic et al. [204] directly tested in a reconstituted system with purified recombinant proteins and defined DNA substrates with representative oxidized lesions (8-oxoguanine or an abasic site) the efficiency of DNA synthesis by the human mitochondrial replisome which included Twinkle helicase. They found substantial stalling at sites of the base lesions by POL γ , even when TWINKLE and mtSSB were present. PrimPol translesion polymerase, which was reported to localize to both nuclei and mitochondria [205], also failed to stimulate oxidative damage bypass by POL γ ; however, Twinkle was able to stimulate PrimPol DNA synthesis on either damaged or undamaged DNA substrates at dNTP levels thought to be characteristic of cycling cells (2 μ M dTTP, 1 μ M dCTP, 2 μ M dATP and 1 μ M dGTP) [204].

The data from biochemical assays have not yet elucidated a direct role of Twinkle helicase in a specific pathway of oxidatively damaged DNA processing; however, results from a transgenic mouse model of overexpressed Twinkle suggested that mtDNA replication stalling at oxidative damage in heterozygous mitochondrial superoxide dismutase knockout hearts could be overcome; moreover, cardiomyopathy was reduced in a p21-dependent manner [206]. In another study with the overexpressed Twinkle transgenic mouse model, mtDNA copy number and cardioprotection was increased in volume overload-induced heart failure [207]. Thus, Twinkle's ability to upregulate mitochondrial biogenesis can help to alleviate oxidative stress associated with cardiac dysfunction. Twinkle's ability to catalyze homologous strand exchange and DNA branch-migration and its proposed role in

recombinational repair and remodeling of stalled mitochondrial replication forks [42, 208] may be relevant to situations of elevated oxidative stress.

Cockayne syndrome, CSB DNA translocase, and its role in the oxidatively damaged DNA response

Cockayne syndrome (CS) is a DNA repair disorder characterized by features of accelerated aging, and linked to hereditary mutations in the *CSA* or *CSB* genes; *CSA* encodes a WD40 repeat protein serving as an adaptor in an E3-ubiquitin ligase complex and *CSB* encodes an ATP-dependent DNA translocase/chromatin remodeling factor (for review, see [209, 210]). *CSA* and *CSB* have been implicated in transcription-coupled NER of bulky adducts; however, they may also play a more general role in the metabolism of transcription-blocking lesions that also arise from oxidative modifications (e.g., cPu, ICL) [211]. Kyng et al. reported from their microarray analysis that CS-B fibroblasts exposed to H₂O₂ displayed a generally reduced expression of >100 genes, particularly those involved in DNA repair, signal transduction, and ribosomal functions [212]. Consistent with these finds, it was earlier reported that the repair of the oxidative lesion 8-oxoG was significantly reduced in CS-B cells due to reduced expression of the *hOGG1* gene that encodes a 8-oxoG DNA glycosylase [213]. Furthermore, Kamenisch et al. showed that CS-B localizes to mitochondria and interacts with the BER-associated human mitochondrial 8-oxoguanine glycosylase-1 in response to oxidative stress [214]. CS-B mutant cells were found to be sensitive to γ -radiation and accumulate 8-oxoG, providing further evidence of a general genome BER defect [215]. Consistent with these findings, global genome repair of 8-oxoG was significantly reduced in a UV61 hamster cell line defective in the gene homologous to human *CSB* [216]. *CSB* is likely to play a direct role in BER of oxidatively damaged DNA as it interacts physically and functionally with several important players in the pathway (*APE1* [217], *NEIL1* [218], and *NEIL2* [219]). CS-A cells were also found to be deficient in repair of 8-oxoG, and expression of an *E. coli* formamidopyrimidine DNA glycosylase corrects the oxidative DNA repair defect in both CS-A and CS-B cells [220].

Given that mitochondria are characterized by an environment of highly elevated ROS, it is reasonable to believe that *CSB* acts to suppress oxidatively damaged DNA in this organelle as well as the nucleus. A role of *CSB* DNA translocase in mitochondria was first reported by the Bohr lab in which they found that repair of mitochondrial 8-oxoG was deficient in cells from CS-B patients [221]. They went on to show in a subsequent study that *CSB* mitochondrial localization is enhanced following cellular exposure to the oxidative agent menadione, and that BER incision of 8-oxoG, uracil, and 5-hydroxyuracil are all reduced in *CSB*-deficient cells [222]. In addition to its involvement in repair of oxidatively damaged mtDNA, *CSB* promotes mitochondrial transcription elongation [223]. An analysis of primary fibroblasts from CS patients revealed that a deficiency in either *CSA* or *CSB* resulted in an altered redox balance accompanied by elevated ROS, increased oxidatively damaged DNA, and decreased mitochondrial membrane potential and oxidative phosphorylation [224]. Scheibye-Knudsen et al. reported elevated metabolism in a *CSB*^{m/m} mouse model and human *CSB*-deficient cells, accompanied by down-regulation of mitophagy, suggesting that *CSB* acts to sense mtDNA damage and promote mitochondrial

autophagy [225]. In other work, Cleaver et al. presented findings indicating that CSB acts to scavenge mitochondrial ROS, which helps to suppress oxidatively damaged DNA [226]. The latest findings from the Bohr lab showed that a high-fat diet, PARP inhibition, or NAD⁺ replenishment rescued a number of CS-associated phenotypes in CSB-deficient mice, pointing toward a role of PARP1 hyper-activation in promoting CS when CSB is deficient [227].

Collectively, the results from CS mice, nematodes, and human cells point toward mitochondrial dysfunction and reduced BER, in addition to defective transcription-coupled NER, as major contributing factors to accelerated aging in CS [209]. It remains to be seen if the neurodegeneration and other clinical features characteristic of CS are largely attributed to an imbalance of ROS in mitochondria which drives accumulation of oxidative damage or if they also arise from defects when CSA/CSB is absent to facilitate transcription and/or repair of nuclear oxidatively damaged DNA. Recently it was shown that an ubiquitylation site in CSB mediates its role in oxidatively damaged DNA repair [228], consistent with an earlier observation that CSB rapidly recruits to sites of oxidatively damaged DNA [229]. CSB localizes to promoters of specific genes in response to oxidative stress in a manner that is regulated by a transcription factor that regulates long-range chromatin interactions, further supporting a model in which CSB's role in transcriptional control as well as DNA repair is important [230]. It seems probable that both nuclear and mitochondrial oxidative stress contributes to CS, extending the function of CSB and CSA beyond their classic role in transcription-coupled NER.

Summary

In this review, we have summarized experimental findings which pertain to how DNA helicases affect the metabolism of oxidatively damaged DNA through their roles in gene expression, replication, and DNA repair. From a review of the literature, it is apparent that a number of the RecQ and Fe-S helicases that play prominent roles in disease and cancer are intimately engaged in the response to oxidative stress through distinct pathways such as BER, telomere maintenance, and mtDNA metabolism. We have discussed the potentially important role of DNA helicases to help cells cope with oxidized lesions that arise in guanine-rich sequences elements, including those that form G-quadruplex DNA structures. Understanding at the mechanistic and biological levels the unique roles of helicases in the metabolism of oxidatively damaged DNA should help to create insights for how these molecular motors and their pathways may be targeted therapeutically as pre-clinical models emerge.

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Highlights

- Sensitivity to oxidized bases depends on helicase, lesion, and strand residence
- Helicases may recognize DNA damage by an electron charge transport mechanism
- Oxidatively damaged G4 DNA may be acted upon by specialized helicases
- Helicases assist in repair of oxidatively damaged mitochondrial DNA

Fig. 1.

Disruption of charge transport through the DNA double helix by alternative DNA structure or an oxidized base may signal redox-active Fe-S DNA repair proteins including helicases to sites of DNA damage. See text for details.

Fig. 2. Guanine-rich DNA sequences in the mitochondrial genome and promoters, ribosomal DNA, telomeric repeats, or other regions of the nuclear genome may be targeted by DNA helicases for unwinding to facilitate DNA transactions such as replication, DNA repair, or transcription. See text for details.

Fig. 3. Oxidative stress may have reciprocal effects on epigenetic regulation, mitochondrial function, inflammation, and DNA damage that lead to progressive bone marrow failure prevalent in the genetic disorder Fanconi Anemia. See text for details.

Table 1

Inhibition of helicase-catalyzed unwinding by replication-blocking lesions.

Helicase	Thymine Glyco	Cyclopurine
RECQL1	++++ (tr. str.) ⁴³	++++ (tr. str.) ⁴¹
WRN	+++ (tr. str.) ⁴³	+++ (tr. str.) ⁴¹
BLM	+++++ (tr. str.) ⁴³	+++++ (tr. str.) ⁴¹
FANCI	+++++ (ntr. str.) ⁴³	
	+++++ (tr. str.) ⁴³	+++ (tr. str.) ⁴¹
DDX11 (CHIR1)	ND	+ (tr. str.) ⁴¹
Twinkle	+++ (tr. str.) ⁴²	– ⁴²

+++++, Strong inhibition; +++, Modest (~2-fold) inhibition; –, no detectable inhibition; **ND**, not determined;**tr. str.**, translocating strand inhibition; **ntr. str.**, non-translocating strand inhibition

Table 2

Mitochondrial oxidative stress associated with deficiencies in DNA helicases.

Helicase	Oxidative Stress Phenotype
XPB	XPB-deficient cells display increased ROS production in mitochondria and are hypersensitive to H ₂ O ₂ -induced oxidative DNA damage ¹⁶⁹ .
RECQL4	RECQL4-deficient cells have increased endogenous DNA damage, increased mitochondrial superoxide production, lower reserve capacity, and elevated aerobic glycolysis-dependent cell invasion ^{171, 173, 176} .
DNA2	DNA2-deficient cells are compromised in their ability to repair mitochondrial oxidative DNA lesions by the BER pathway ^{179, 180} .
PIF1	To our knowledge, the status of PIF1 deficiency on oxidative DNA damage processing in human cell has not been determined.
SUV3	No direct role of SUV3 deficiency on oxidative DNA damage processing in human cells has been determined; however, SUV3 belongs to a protein complex that modulates messenger RNA polyadenylation in response to cellular energy changes ¹⁹⁸ .
Twinkle	Overexpressed Twinkle in a transgenic mouse model suppresses symptoms induced by oxidative stress ^{205,206} .
CSB *	CSB-deficient cells display reduced mitochondrial BER ^{212, 219, 220} , altered redox balance and elevated ROS ^{213, 214, 215} . CSB-deficient mice and human cells display reduced mitophagy ²¹⁴ . PARP1 is hyper-activated in CSB-deficient mice ²¹⁶ .

* CSB contains the conserved ATPase/helicase motifs and is an ATP-dependent DNA translocase, but not a bona fide helicase.