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REVIEW

Functional interplay between ATM/ATR-mediated DNA damage response and DNA repair pathways in oxidative stress

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Abstract To maintain genome stability, cells have evolved various DNA repair pathways to deal with oxidative DNA damage. DNA damage response (DDR) pathways, including ATM-Chk2 and ATR-Chk1 checkpoints, are also activated in oxidative stress to coordinate DNA repair, cell cycle progression, transcription, apoptosis, and senescence. Several studies demonstrate that DDR pathways can regulate DNA repair pathways. On the other hand, accumulating evidence suggests that DNA repair pathways may modulate DDR pathway activation as well. In this review, we summarize our current understanding of how various DNA repair and DDR pathways are activated in response to oxidative DNA damage primarily from studies in eukaryotes. In particular, we analyze the functional interplay between DNA repair and DDR pathways in oxidative stress. A better understanding of cellular response to oxidative stress may provide novel avenues of treating human diseases, such as cancer and neurodegenerative disorders.

Keywords AP sites · Base excision repair · Homologous repair · Mismatch repair · Nucleotide excision repair · Oxidative stress-induced DNA damage · Reactive oxygen species

Abbreviations

9-1-1 complex	Rad9-Rad1-Hus1
AP	Apurinic/apyrimidinic
APE1	AP endonuclease 1
APE2	AP endonuclease 2
A-T	Ataxia-telangiectasia
ATM	A-T mutated
ATR	ATM- and Rad3-related
BER	Base excision repair
Chk1	Checkpoint kinase 1
Chk2	Checkpoint kinase 2
DDR	DNA damage response
DSB	Double-strand break
GG-NER	Global genome NER
HR	Homologous recombination
Ku complex	Ku70/Ku80
MCM	Minichromosome maintenance
MMR	Mismatch repair
MRN complex	Mre11-Rad50-Nbs1
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
PCNA	Proliferating cell nuclear antigen
ROS	Reactive oxygen species
RPA	Replication protein A
SSB	Single-strand break
SSBR	SSB repair
ssDNA	Single-stranded DNA
TC-NER	Transcription-coupled NER
TDP1	Tyrosyl-DNA phosphodiesterase 1
γ-H2AX	H2AX phosphorylation at Serine 139

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Oxidative stress-induced DNA damage

Oxidative stress

Cells of all organisms are constantly exposed to insults such as oxidative stress from endogenous and exogenous

sources. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses [1–4]. ROS include, but are not limited to, the oxygen molecule (O_2), superoxide anion radical ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and singlet oxygen ($^1O_2$) [5, 6]. ROS are generated endogenously from normal cellular metabolism such as oxidative phosphorylation in mitochondria and long-chain fatty acids oxidation in peroxisomes [7]. ROS are also formed by exogenous sources such as ionizing radiation (IR), ultraviolet (UV) radiation, chemotherapeutic agents, and environmental agents [7–10]. Representing a major threat to cells, ROS may react with almost all macromolecules including DNA, RNA, proteins, and lipids.

To protect themselves against ROS, cells have evolved several antioxidant defense programs. Antioxidants and protein scavengers can detoxify ROS [11]. Antioxidants include low molecular weight vitamin E (α-tocopherol), vitamin C (ascorbic acid), uric acid, glutathione, β-carotene, and ubiquinone, whereas examples of protein scavengers are hemoglobin and ferritin [11–13]. Furthermore, a variety of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione-S-transferase, can facilitate reduction reactions of ROS. However, ROS generation periodically exceeds antioxidant defense capacity, leading to oxidative stress in cells. Oxidative stress is implicated in the pathogeneses of cancer, aging, neurodegenerative disorders, diabetes mellitus, obesity, heart failure, cardiovascular diseases, inflammation, ischemia/reperfusion injury, kidney injury/failure, obstructive sleep apnea, and hypertension [14, 15]. Research into oxidative stress is attracting attention of most biomedical disciplines, including both basic and translational research, as evidenced by approximately 121,000 search results in PubMed® using "oxidative stress" as key words.

Oxidative stress-induced DNA damage

"Oxidative DNA damage" is widely used in the literature to describe oxidative stress-induced DNA lesions, whereas "oxidatively damaged DNA" is also recommended [16]. It is estimated that oxidative stress may induce approximately 10,000 DNA alterations per cell per day, representing a major portion of endogenous DNA damage [17–20]. Oxidative stress can induce a variety of different types of DNA damage or replication stress, such as base (purine and pyrimidine) damage, sugar moiety damage, Apurinic/apyrimidinic (AP) sites, DNA single-strand breaks (SSBs), DNA double-strand breaks (DSBs), tandem base modifications (e.g., DNA intrastrand crosslink), DNA interstrand crosslinks, protein–DNA crosslinks, mismatched pairs with damaged bases, stalled DNA replication forks, and oxidatively-generated clustered DNA lesions (OCDLs) [17–21]

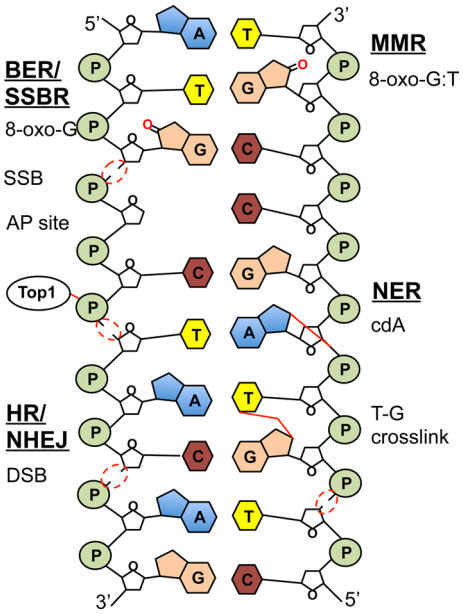

Fig. 1 Oxidative DNA damage and DNA repair pathways involved in oxidative stress. Base lesion [e.g., 8-oxo-G (8-oxoguanine)], AP site, SSB, and protein–DNA crosslink (e.g., Top1-DNA crosslink) are primarily repaired by BER/SSBR. Mismatched pairs with damaged bases (e.g., 8-oxo-G:T mismatch pairs) are repaired by MMR. NER is involved in removing tandem lesions (e.g., 8,5′-cyclo-2′-deoxyadenosine (cdA) and T-G intrastrand crosslink), whereas DSB is fixed by HR or NHEJ. *Dashed circles in red* highlight SSB and DSB

(Fig. 1). Hydroxyl radicals can react with the purines and pyrimidines of DNA by the addition of double bonds and the abstraction of a H atom ($H^{\cdot}$) from the methyl group of thymine and from each of the C–H bonds of 2′-deoxyribose. This results in products, such as 5-hydroxymethyl-uracil, C8–OH–adduct radical of guanine, and 8-hydroxyguanine (8-OH-G) [22]. Hydroxyl radicals sometimes target each C atom of the DNA sugar moiety, generating a variety of products, such as 2-deoxypentose-4-ulose, 2-deoxypentonic acid lactone, erythrose, 2-deoxytetradialdose, glycolic acid, and AP sites [9]. Singlet oxygen reacts with all four deoxynucleotide bases with guanine as its preference, generating 8-oxo-7,8-dihydroguanine (8-oxo-G) as the major product [5]. Intrastrand crosslinks, interstrand crosslinks, DSBs, and OCDLs can be formed after exposure to IR and UV radiations [21]. During DNA replication, an oxidatively damaged site may be bypassed by incorporating a mismatched deoxynucleotide (e.g., 8-oxo-G:A) [7]. Chemotherapeutic agents can generate oxidative DNA damage. For example, artesunate can induce 1,N6-ethenoadenine, SSBs and replication-associated DSBs [23–25]. Oxidative stress by hypoxia-reoxygenation in endothelial cells may induce more profound damage, such as chromosomal aberrations (e.g., dicentric chromosomes) and micronuclei [26].

To measure oxidative stress-induced DNA damage, a number of analytical and biochemical methods have been developed, including gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) derived approaches (HPLC–ECD, HPLC–MS/MS, HPLC/MS, and HPLC/^{32}P-postlabeling), immunoassays, and enzymatic assays (comet and alkaline elutions) [27]. Immunofluorescence analysis was used to examine representative oxidative DNA damage 8-oxo-G [28]. Comet assays (single cell gel electrophoresis) were used to measure oxidative stress-induced DNA damage in human lymphocytes from different donors [29]. The Comet-FISH (fluorescence in situ hybridization) technique is used to detect overall and region/site-specific DNA lesions induced by oxidative stress at the individual cell level [30]. However, even with a plethora of techniques, it remains a challenge to measure repair intermediates during the processing of oxidatively damaged DNA.

DNA damage response pathways in oxidative stress

In response to oxidative DNA damage, various DNA repair and DNA damage response (DDR) pathways are employed by cells to maintain genomic integrity [12, 18, 19, 31] (Figs. 1, 2). Base excision repair (BER)/single-strand break repair (SSBR) (Fig. 3), nucleotide excision repair (NER) (Fig. 4), mismatch repair (MMR) (Fig. 5), homologous recombination (HR), and nonhomologous end joining (NHEJ) (Fig. 6) are all involved in the repair processes in response to oxidative DNA damage [7, 18]. Ataxia-telangiectasia mutated (ATM)-Checkpoint kinase 2 (Chk2) and ATM- and Rad3-related (ATR)-Checkpoint kinase 1 (Chk1) checkpoints are the two major DDR pathways induced by oxidatively damaged DNA to coordinate DNA repair process and cell cycle progression (Figs. 2, 7) [31, 32]. Defective DNA repair and DDR pathways may lead to several human diseases, such as cancer and neurodegenerative diseases [6, 33–38] (Fig. 2). To maintain genome stability, the DDR pathways are conserved surveillance mechanisms coordinating DNA repair, cell cycle progression, transcription, apoptosis, and senescence [19, 39–41]. The function of DDR pathways was originally thought to be the alerting of cells to the presence of DNA damage and to arrest cell cycle progress, thus providing extra time for cells to repair DNA damage, such as oxidative DNA damage [31, 42, 43]. The most recent findings are highlighted here regarding how the ATM-Chk2- and ATR-Chk1-dependent DDR pathways are activated in response to oxidative stress.

ATM-Chk2 checkpoint activation in oxidative stress

ATM-Chk2 dependent DDR is activated primarily in response to DSBs (Fig. 7) [44]. If not repaired, DSBs can lead to chromosomal aberrations and the dysfunction of key proteins for cell survival or viability [45–47]. Therefore, DSBs are believed to be one of the most detrimental types of DNA damage for cells [46]. ATM is a defective gene in Ataxia-telangiectasia (A-T), an autosomal recessive disorder with early onset progressive cerebellar ataxia, oculocutaneous telangiectasia, and lymphoid tumor susceptibility [48, 49]. Under normal conditions, ATM is inactive in a dimer or higher-order oligomer status, when its kinase domain is bound by a region surrounding a critical Serine 1981 and prevented from activation. However, in response to DSBs, ATM auto-phosphorylates Serine 1981, leading to dimer dissociation into a monomer and the full activation of ATM [50, 51]. The MRN complex (Mre11-Rad50-Nbs1) is involved in ataxia-telangiectasia like disease and Nijmegen breakage syndrome and is required for ATM activation in DSB response [52–54]. Acting as a DSB sensor, the MRN complex binds to DSBs and recruits ATM to the broken DNA ends for activation. The unwinding of DSB ends by MRN is also important for ATM stimulation [55, 56]. An in vitro reconstitution analysis further revealed that the MRN complex stimulates ATM kinase activity toward its substrates such as Chk2, p53, and histone H2AX [57] (Fig. 7).

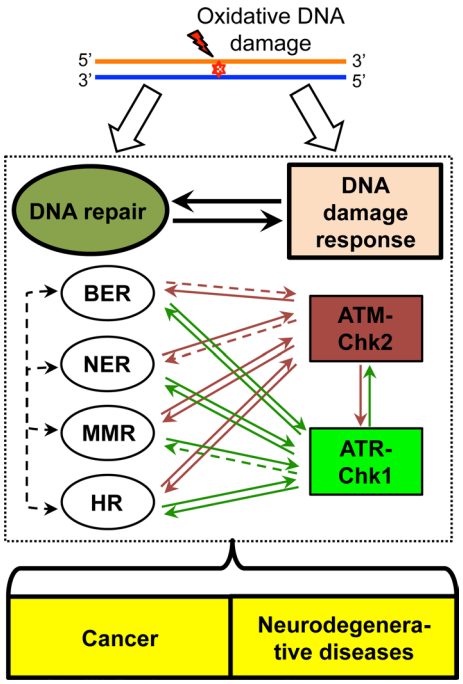

Fig. 2 Cellular responses to oxidative DNA damage. DNA repair pathways (BER, NER, MMR, and HR) and DNA damage response pathways (ATM-Chk2 and ATR-Chk1) are integrating into an interacting network in response to oxidative stress. *Dashed arrows* indicate that potential regulations require more investigations. Defective DNA repair and DDR pathways may lead to diseases such as cancer and neurodegenerative diseases

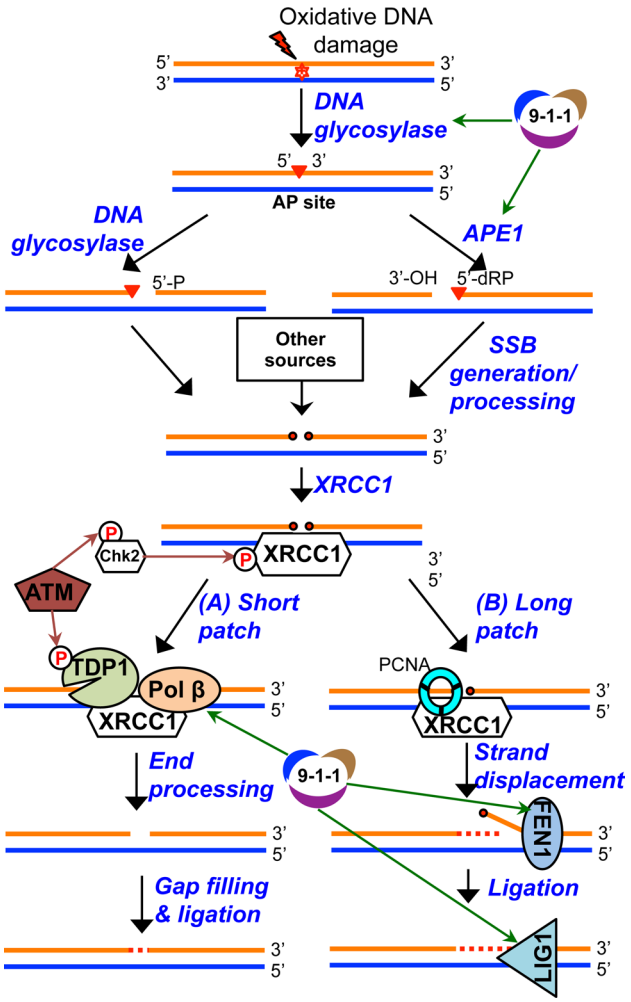

Fig. 3 ATM/ATR pathways promote BER/SSBR in response to oxidative DNA damage. AP site is formed after removal of damaged base by DNA glycosylase. SSBs are generated by APE1 at the 5′ side of AP site or bifunctional DNA glycosylase at the 3′ side of AP site, whereas SSBs may also be from other sources. SSBs can be recognized and bound by scaffolding protein XRCC1. *A* In the short patch sub-pathway, SSB is processed by Pol β to form 1 nt gap. The gap is filled and the final nick is sealed. TDP1 is in charge of removing Topoisomerase I from the protein–DNA crosslink. ATM phosphorylates TDP1 and Chk2. Chk2 then phosphorylates XRCC1. *B* In the long patch sub-pathway, the 3′ side of SSB is extended by PCNA-tethered DNA polymerases when the 5′ side of SSB can't be processed into the normal 5′-phosphate. A short strand (~2–13 nt) at the 5′ side of SSB is displaced and further cleaved by PCNA-mediated FEN1. The subsequent nick in the long patch sub-pathway is finally sealed by LIG1. The 9-1-1 complex stimulates enzyme activities of DNA glycosylase, APE1, Pol β, FEN1, and LIG1

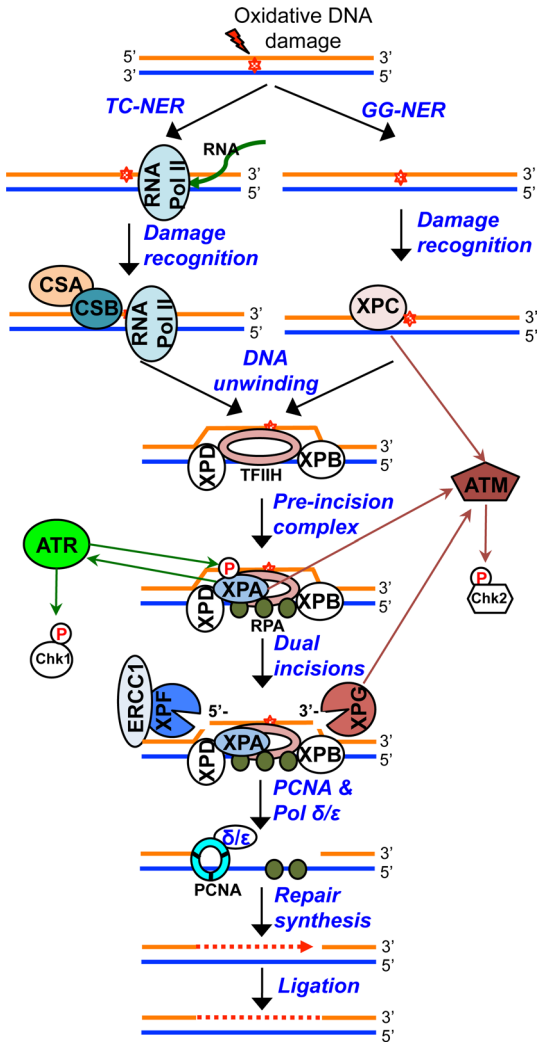

Fig. 4 ATM/ATR pathways interact with NER in response to oxidative DNA damage. In TC-NER, RNA Pol II stops at helix-distorting DNA lesions, which is recognized by CSA/CSB. In GG-NER, the damaged nucleotides are recognized by XPC. The fragment containing the damaged nucleotides is unwound by XPB/XPE together with TFIIH. A pre-incision complex is formed after RPA-mediated XPA recruitment. Dual incisions are achieved at the 5′ side by ERCC1/XPF and at the 3′ side by XPG. Repair synthesis is achieved by the PCNA-mediated DNA Pol δ/ε and followed by ligation via LIG3. XPC, XPA, and XPG regulate ATM directly, whereas XPA also regulates ATR. ATR phosphorylates XPA directly, which is required for the nuclear import and stability and XPA

Several mediator proteins are involved in the regulation of ATM activation. As an ATM substrate, histone H2AX is phosphorylated at Serine 139, which is referred to γ-H2AX and prevents DSBs from processing into chromosomal translocations [58, 59]. 53BP1 (p53 binding protein 1) is a tumor suppressor that colocalizes with γ-H2AX and plays an early role in the response to DSBs [60]. With a forkhead-associated (FHA) domain and two BRCT domains, MDC1 is phosphorylated by ATM and relocalizes to damage sites that contain the MRN complex, γ-H2AX, and 53BP1, thereby playing an indispensable role in ATM-dependent DNA damage checkpoint [61]. MDC1 directly binds to γ-H2AX via its BRCT domain to regulate cellular response to DSBs [62, 63]. DDR signaling also utilizes post-translational modifiers, such as

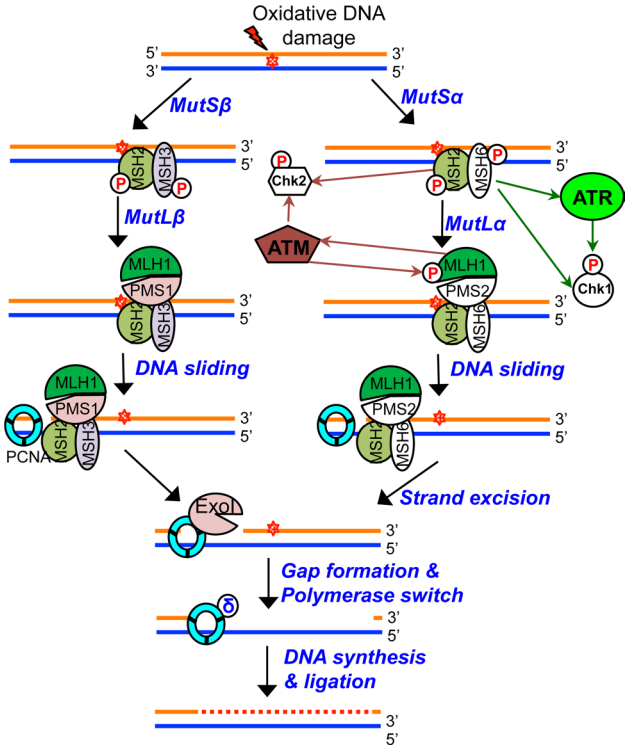

Fig. 5 MMR crosstalks with ATM/ATR pathways in response to oxidative DNA damage. Mismatch pairs with damaged base are recognized by MutSα (MSH2 and MSH6) and MutSβ (MSH2 and MSH3), which are required for the binding of MutLα (MLH2 and PMS2) and MutLβ (MLH2 and PMS1), respectively. MutLα/MutLβ may slide away from mismatch pairs and create a nick by the endonuclease activity. The strand containing the mismatch pair may be excised by nucleases such as Exo1 in a PCNA-dependent manner. Pol δ/ε will switch back to fill the gap, and the final nick is sealed by DNA ligase. MSH2, MSH3, and MSH6 may be phosphorylated by ATM/ATR. MSH2 associates with Chk2 while ATM associates with MLH1. ATM phosphorylates MLH1. MSH2 recruits ATR and Chk1 to damaged sites

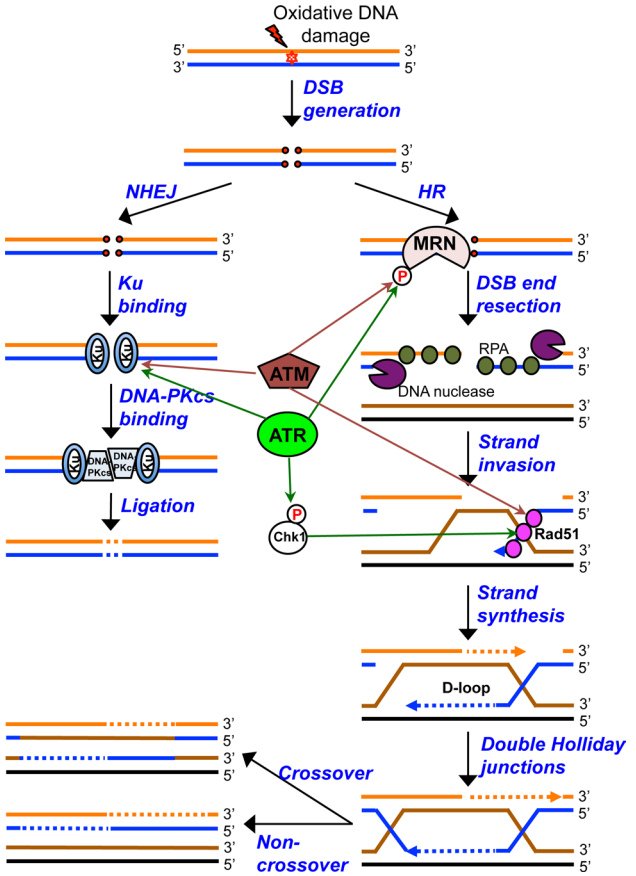

Fig. 6 ATM/ATR pathways regulate HR and NHEJ in response to oxidative DNA damage. DSBs can be resected by DNA nuclease, generating RPA-coated ssDNA. Rad51-coated filaments invade the homologous strand and strand synthesis continues to form the D loop. The Double Holliday junctions are resolved to generate crossover or non-crossover products. The Ku complex (KU70/Ku80) is bound to both DSB ends with the absence of homologous chromosome in the NHEJ pathway. The Ku complex is also regulated by ATM/ATR. Subsequently, the DSB ends are processed by the catalytic subunits of DNA-PK (DNA-PKcs) and the broken ends of DSB are finally ligated. The MRN complex at the site of DSB may be phosphorylated by ATM/ATR, whereas ATM may also regulate Rad51

ubiquitination and sumoylation, as molecular switches to regulate cellular responses to DSBs [64, 65]. RNF4, a SUMO-targeted ubiquitin E3 ligase, is recruited to DSBs by MDC1 and promotes DNA repair and cellular response to DSBs [66, 67].

ATM appears to be activated in oxidative stress response. In an ischemic retinopathy model, ATM activation by ROS promotes endothelial proliferation by suppressing ROS accumulation as a feedback mechanism [68]. Hydrogen peroxide treatment in primary neuron cells triggers γ-H2AX, indicating DSB generation and ATM activation [69]. Hydrogen peroxide induces p53 phosphorylation at Serine 15 and cell cycle arrest in an ATM-dependent manner in human umbilical vein endothelial cells [70]. Reoxygenation-induced oxidative DNA damage also triggers p53 phosphorylation at Serine 15 [71]. Ochratoxin A, one of the most abundant mycotoxin food contaminants, induces oxidative DNA damage and triggers an ATM-dependent G2 arrest in human gastric epithelium GES-1 cells [72].

As A-T fibroblast cells ($ATM^{-/-}$) are more susceptible to oxidative stress than normal cells, ATM was proposed as a major sensor of oxidative DNA damage or ROS [73]. Notably, ATM was directly activated by hydrogen peroxide-induced oxidation in the absence of DNA damage, evidenced by ATM auto-phosphorylation at Serine 1981, p53 phosphorylation at Serine 15, and Chk2 phosphorylation at Threonine 68, but not by H2AX phosphorylation at Serine139 [74–76]. Hydrogen peroxide represses mTORC1 in a dose and time-dependent manner, and the mTORC1 repression by ROS requires the presence of ATM in the cytoplasm, suggesting a sub-cellular compartment

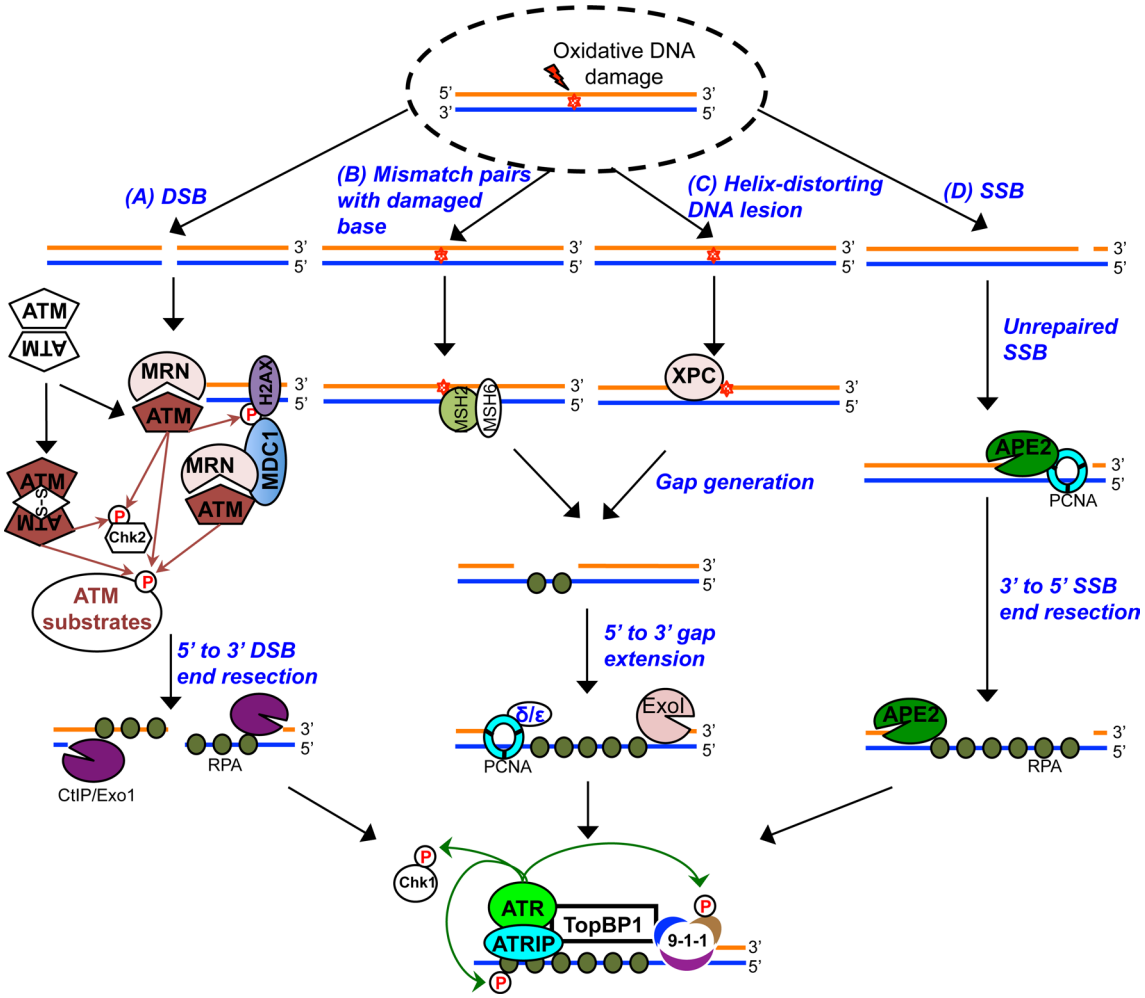

Fig. 7 ATM/ATR pathways in response to oxidative stress. *A* Oxidative stress can induce dimerization of ATM via its cysteine residues, leading to structural change in ATM protein and elevated ATM kinase activity. After dissociating from its homodimer, ATM can associate with the MRN complex, which localizes at oxidative stress-derived DSBs, or ATM can associate with MDC1. H2AX is localized to the flanking region of DSB and its phosphorylation mediates the recruitment of MDC1. Activated ATM then phosphorylates its substrates including Chk2. Long stretch of ssDNA is generated through DSB end resection in the 5′–3′ direction via DNA nucleases such as CtIP and Exo1. *B* Oxidative stress-induced mismatch pairs with base lesion are recognized by MSH2-MSH6 complex. *C* The helix-distorting DNA lesions are recognized by XPC in GG-NER and further incised by dual enzyme complexes including XPG. A gap of ssDNA can be generated in MMR or NER pathways and extended in the 5′–3′ direction by end resection via DNA nucleases such as Exo1. D Unrepaired SSBs may be processed by PCNA-dependent APE2 in the 3′–5′ direction, generating RPA-coated ssDNA. ATR/ATRIP and the 9-1-1 complex are recruited to RPA-ssDNA independently. TopBP1 bridges the ATR/ATRIP and the 9-1-1 complex and activates ATR kinase directly. Then activated ATR phosphorylates its own downstream substrates, such as Chk1, RPA32, and Rad1

requirement for ATM signaling in oxidative stress [77]. ATMIN, an ATM interactor protein, was identified as an essential component in the ATM checkpoint pathway in response to oxidative stress. ATMIN mediates oxidative stress-induced ATM activity, thereby protecting the aging brain from accumulating DNA damage [78].

ATR-Chk1 checkpoint activation in oxidative stress

Whereas the ATM-Chk2 checkpoint pathway is activated primarily by DSBs, the ATR-Chk1 checkpoint pathway is activated by replication stress or other types of DNA damage (Fig. 7) [79]. ATR was originally cloned as a member of the phosphoinositide 3-kinase–related kinase (PIKK) family and is essential for early embryonic development [32, 80, 81]. Aberrant expression of ATR results in Seckel syndrome, an autosomal recessive disorder associated with growth retardation and microcephaly [82]. An activated ATR kinase phosphorylates a number of downstream substrates, which are involved in nucleic acid metabolism (DNA replication, DNA repair, DNA recombination, mRNA transcription, and RNA processing), protein

metabolism, and cell cycle control [83, 84]. As a critical player in DDR, Chk1 is phosphorylated at Serine 345 by ATR in response to stalled DNA replication forks and DNA damage induced by UV, IR, methyl methanesulfonate (MMS), mitomycin C (MMC), and hydrogen peroxide [85–90]. The phosphorylation of Chk1 enhances Chk1's kinase activity, which in turn phosphorylates downstream substrates (e.g., Cdc25, BLM, and FANCD2/FANCE) and facilitates cell cycle arrest and DNA damage repair [91–93].

The molecular mechanisms for ATR activation are very complex although a canonical ATR checkpoint signaling pathway has been proposed [94]. 5′-primed ssDNA coated with RPA (RPA-ssDNA) is the determinant DNA structure for ATR activation in response to stalled DNA replication forks or DNA damage [41, 94–96]. A long stretch of ssDNA can be generated through the functional uncoupling of minichromosome maintenance (MCM) helicase and DNA polymerase in DNA replication stress, the DSB end resection by DNA exonuclease such as CtIP in DSB response, the Exo1-mediated processing of NER intermediates after UV radiation, and the SSB end resection by AP endonuclease 2 (APE2) in oxidative stress [86, 97–99] (Fig. 7). ATR-interacting protein ATRIP is recruited to ssDNA via direct interaction between RPA and ATRIP, thereby recruiting ATR to RPA-ssDNA [100, 101]. The PCNA-like clamp 9-1-1 complex (Rad9-Rad1-Hus1) is recruited preferentially to the 5′-primed ssDNA/dsDNA junction by RPA and the clamp loader Rad17-RFC complex [102–105]. The ATR-ATRIP complex and 9-1-1 complexes are recruited to RPA-ssDNA independently and are bridged by the multiple-function protein TopBP1 (Topoisomerase IIβ binding protein 1) [106–110]. TopBP1 contains nine BRCT domains and is required for DNA replication initiation via the recruitment of CDC45 [111, 112]. In response to DNA damage or replication stress, TopBP1 associates with ATR and ATRIP by its C-terminus and Rad9 of the 9-1-1 complex via its N-terminal BRCT domains, which in turn directly activates ATR kinase activity [113–118]. The roles of TopBP1 in DNA replication and DDR can be functionally separated through their distinct BRCT domains [110, 119, 120]. TopBP1 is required for the recruitment of ATR and the 9-1-1 complex onto genotoxin-damaged chromatin or stalled replication forks in *Xenopus* egg extracts and human cell lines [121–123]. The recruitment of TopBP1 to stalled replication forks or DSBs may also require MDC1/H2AX and the MRN complex [124–126]. Claspin, a Chk1-binding protein, is required for ATR-Chk1 checkpoint activation [127–129]. The circadian protein Tim (Timeless) -Tipin (Timeless-interacting protein) complex associates with ATR-Chk1 checkpoint proteins and plays an essential role in DDR as well [130–133]. After DNA damage is repaired or a pathway selection has been made, the ATR-Chk1 checkpoint will be terminated using checkpoint adaption or a recovery mechanism to resume normal cell cycle progression, which may require Plk1-mediated phosphorylation and subsequent degradation of Claspin [134, 135].

New evidence has been shown that ATR-Chk1 checkpoint signaling is also triggered by oxidative stress. Hyperoxic conditions (95 % oxygen versus the normal 21 % oxygen) resulted in the phosphorylation of Chk1 (Serine 345) and p53 (Serine 15, Serine 37, and Serine 392) in an ATR-dependent but ATM-independent fashion in the lung adenocarcinoma cell line A549 [136]. More recently, it has been demonstrated that hydrogen peroxide triggers ATR-Chk1 checkpoint signaling in human dermal fibroblasts HDF cells [137] and in *Xenopus* egg extracts [86]. In addition, the natural antioxidant Lycopene inhibits *H. pylori*-induced gastric diseases associated with oxidative DNA damage (e.g., 8–OH–G and DSBs) and prevents ROS-induced ATM- and ATR-mediated DDR in gastric epithelial AGS cells [138].

Crosstalk between ATM-Chk2 and ATR-Chk1 pathways

It was originally proposed that the ATM-Chk2 and ATR-Chk1 checkpoint pathways work independently in response to different types of replication stress or DNA damage [79]. However, there is a suggestion of crosstalk and transition between these two pathways. In DSB end resection, ATM and the MRN complex are both required for the generation of RPA-ssDNA, the important DNA structure for ATR activation. ATM is essential for the recruitment of ATR to IR-damaged chromatin and Chk1 phosphorylation at Serine 345 [139]. Furthermore, ATM is essential for DSB-induced ATR activation in the S and G2 phases of cell cycle, but not in the G1 phase [140]. Additionally, an ATM-to-ATR switch during the biphasic DSB response was proposed in a report using mammalian cell lysates [141]. Notably, ssDNA may regulate the ATM-to-ATR switch in an ATM and DSB end resection-dependent fashion. Therefore, the DNA end structure determines the molecular mechanism of activating ATM-Chk2 and ATR-Chk1 pathways in an orderly fashion [142].

Alternatively, in response to the DNA methylating agent temozolomide, the ATR-Chk1 checkpoint is activated to arrest cell cycle at G2/M transition. This is followed by ATM-Chk2 checkpoint activation, suggesting ATR functions earlier than ATM [143]. ATM auto-phosphorylation at Ser1981 is ATR-dependent and ATM-independent in response to stalled DNA replication forks or UV-induced DNA damage [144]. In contrast to the IR situation, UV-induced ATM phosphorylation at Serine 1981 does not require the MRN complex, which is a requirement for ATM

activation by DSBs. ATR-dependent ATM activation promotes Chk2 phosphorylation downstream of the checkpoint signaling [144]. UV-induced replication stress triggers ATM phosphorylation at Serine 1981, Chk2 phosphorylation at Threonine 68, and H2AX phosphorylation at Serine 139. Further time-dependent experiments revealed that ATM phosphorylation and Chk2 phosphorylation peak after Chk1 phosphorylation. This is consistent with the observation that DSBs are observed in the late phase in response to stalled DNA replication forks [145]. These findings suggest that ATM and ATR may be activated sequentially, the order of which is dependent on the nature of DNA damage or stress (DSBs versus stalled replication forks or UV radiation), and that there is direct cross talk between the two key DDR kinases.

ATM and ATR checkpoint pathways are activated simultaneously by ROS-induced DNA damage in human monocytes [146]. 4-Hydroxynonenal (HNE)-induced DSBs enhance comet tail formation and H2AX phosphorylation in hepatocellular carcinoma cells, suggesting ATM activation [147–149]. HNE also induces caffeine-sensitive ATR phosphorylation at Serine 428 and Chk1 phosphorylation at Serine 296 [147]. Artesunate can cause oxidative DNA damage and trigger the phosphorylation of ATM, Chk2, Chk1, and H2AX, and eventually may lead to apoptosis in human glioblastoma cells [25]. Hydrogen peroxide triggers both ATM phosphorylation at Serine 1981 and Chk1 phosphorylation at Serine 344 in *Xenopus* egg extracts [86]. However, eliminating ATM kinase activity by its specific inhibitor KU55933 does not affect hydrogen peroxide-induced Chk1 phosphorylation, suggesting ATM may be nonessential for ATR-Chk1 checkpoint activation in oxidative stress response [86]. Together, it remains unclear how ATM and ATR checkpoint pathways regulate each other in response to oxidative stress.

Functional interplay between DNA damage response pathways and DNA repair pathways in oxidative stress

In response to oxidative stress, DDR pathways not only arrest cell cycle progression, but also directly participate in and facilitate DNA repair pathways. Additionally, DNA repair proteins may sense oxidative DNA damage and process such damage into appropriate structures for DDR activation. Thus, DNA repair and DDR pathways are integrated into interacting networks in response to oxidative DNA damage (Fig. 2) [150]. This review focuses on ATM/ATR-mediated DDR pathways and their connections with DNA repair pathways in response to oxidative stress in hopes of providing a comprehensive perspective on this topic.

DNA repair pathways of oxidatively damaged DNA

To repair oxidative DNA damage, several DNA repair pathways, including BER/SSBR, NER, MMR, HR, and NHEJ, are employed by cells to maintain genome stability (Fig. 1) [7, 18]. DNA repair dysfunction was recently proposed to go from a cancer driver to a therapeutic target [151]. These different repair pathways are also integrated with other cellular processes, including cell cycle control, transcription, and replication, suggesting the presence of a DNA repair network used to prevent and repair oxidative DNA damage [152]. Embedded figures provide schematic representations of DNA repair pathways, which highlight the connections with ATM-Chk2 and ATR-Chk1 pathways (Figs. 3, 4, 5, 6). More details of individual DNA repair pathways may be found from other recently published reviews [7, 9, 153–165].

DDR pathways affect DNA repair pathways

It was proposed recently that DDR kinases promote efficient DNA repair by directly regulating the DNA repair machinery, changing the local chromatin environment and cellular environment [166]. Several studies support the role of ATM-Chk2 and ATR-Chk1 pathways for BER, NER, MMR and HR pathways in cellular response to oxidative stress; however, molecular mechanisms of how exactly DDR pathways regulate DNA repair pathways in oxidative stress require more clarity.

ATM-Chk2 checkpoint pathway facilitates DNA repair pathways

Compelling evidence suggests that DDR components participate in and promote the BER pathway, thereby playing a direct role in DNA repair [167]. Chk2-dependent XRCC1 phosphorylation at Threonine 284 promotes the recruitment of XRCC1 to the initial lesion site recognized and excised by the DNA glycosylases MPG and UNG2 (Fig. 3) [168]. Tyrosyl-DNA phosphodiesterase 1 (TDP1) hydrolyzes the phosphodiester bond at a DNA 3′-end linked to a tyrosyl moiety at Topoisomerase I (Top1)-DNA covalent complexes (Fig. 1). TDP1 protects against oxidative DNA damage and has been implicated in SSBR [169]. ATM-dependent TDP1 phosphorylation at Serine 81 facilitates its recruitment to damage sites via XRCC1, thereby promoting cell survival and DNA repair in the human colon carcinoma cell line HCT116 (Fig. 3) [170]. TDP1 also has 3′-phosphoglycolate excision activity, which may contribute to its participation in DSB repair [171].

The ATM-Chk2 pathway is also implicated in the regulation of MMR pathway. The selenium compound and

its metabolites (e.g., Na_2SeO_3, MSeA, or MSeC) induce ROS, such as 8-oxo-G, and cause MLH1-mediated and G_2/M arrest in HCT116 [172]. Notably, ATM is required for the selenium-induced MLH1-PMS2 association, which is essential for the repair efficiency [172] (Fig. 5). ATM is essential for MLH1 phosphorylation at Serine 406 and its stability following chemotherapy drug doxorubicin treatment, suggesting the regulation of MMR proteins by ATM [173]. Moreover, ATM plays an important role for the recruitment of Ku complex to hydrogen peroxide-damaged chromatin, further supporting the role of ATM in DSB repair by NHEJ [174, 175] (Fig. 6). In addition, systematic analyses of ATM/ATR substrates have revealed several IR-induced phosphorylation in MMR (e.g., MSH2, MSH3, and MSH6) and HR (e.g., Mre11, Rad50, MDC1, and Rad51) proteins [83] and UV-induced phosphorylation in HR proteins (e.g., Mre11, Rad50, and MDC1) [84] (Figs. 5, 6). It has been demonstrated that Mre11 phosphorylation by ATM/ATR may participate in the checkpoint recovery following DSB repair [176]. Conversely, it remains largely unknown exactly how NER proteins are regulated by ATM pathway (Fig. 2).

ATR-Chk1 checkpoint pathway regulates DNA repair pathways

As a critical component of ATR-Chk1 checkpoint pathway, the 9-1-1 complex interacts with several BER proteins: APE1, Polymerase β (Pol β), FEN1, DNA ligase I, as well as DNA glycosylases MutY, NEIL1, and TDG, thereby stimulating their DNA repair activities [177–185] (Fig. 3). Stimulation by the 9-1-1 complex was unique to Pol β as the 9-1-1 has no effect on replicative DNA polymerases δ and ε. Thus, there is a distinct regulatory role for the 9-1-1 complex in BER pathway. Importantly, the recruitment of the 9-1-1 complex to H_2O_2-damaged chromatin requires APE2-mediated generation of RPA-ssDNA, suggesting the 9-1-1 complex may promote the BER pathway via a positive feedback mechanism [86]. A PCNA-like toroidal shape and a single repair enzyme-binding site were revealed from the crystal structure of the 9-1-1 complex [186]. However, it remains elusive how the 9-1-1 complex exactly stimulates the BER pathway at almost all steps. More investigations are also needed to test whether the kinase activity of ATR or Chk1 plays a direct role in the regulation of BER pathway.

In response to UV damage, ATR phosphorylates XPA at Serine 196 and regulates the nuclear import of XPA, suggesting that ATR checkpoint modulates the cellular activity of NER pathway [187, 188] (Fig. 4). Further analysis demonstrated that ATR kinase is indeed required for the GG-NER (global genome-NER) of UV-induced damage, such as 6–4 photoproducts (6–4PPs) and cyclobutane pyrimidine dimers (CPDs) [189]. ATR-dependent phosphorylation of XPA at Serine 196 interferes with its binding to HERC2, thereby antagonizing HERC2-mediated ubiquitination and degradation of the XPA protein [190]. Cisplatin-induced nuclear import of XPA is also dependent on an ATR checkpoint in p53-proficient, but not p53-deficient, lung cancer cells [191]. Therefore, ATR kinase has been proposed as a master regulator of NER in S phase [192]. Additionally, activated Chk1 phosphorylates its own substrates such as protein Rad51, which is involved in the DSB repair by HR [193–195] (Fig. 6).

DNA repair proteins are important for DNA damage response in oxidative stress

DNA repair proteins (such as DNA nucleases) may process DNA damage into structures for DDR protein recruitment or recruit DDR proteins to damaged sites via direct protein–protein interactions. This suggests DNA repair proteins may regulate the DDR pathway directly or indirectly [99, 196, 197]. Oxidative stress is emphasized below (Fig. 7).

BER proteins regulate DDR pathways

In the absence of TDP1, unrepaired oxidative DNA damage triggers an ATR/ATM-dependent apoptotic-like response. This infers that ROS under physiological quiescent conditions represent a detrimental threat to genomic stability [198]. Consistent with this observation, MMS induces unresolved BER intermediates in XRCC1-deficient cells and activates ATM and ATR-dependent DDR pathways, including S-phase delay and SMC1 (structural maintenance of chromosomes protein 1) phosphorylation at Serine 966 and Chk1 phosphorylation at Serine 345 [199]. However, it remains elusive whether and how the BER pathway affects DDR directly. A recent study shows that a BER protein APE2 plays several essential roles for ATR-Chk1 checkpoint signaling during oxidative stress [86]. APE2 binds to hydrogen peroxide-damaged chromatin and resects the SSB in the 3′–5′ direction via its 3′-phosphodiesterase and 3′–5′ exonuclease activities, generating RPA-ssDNA. Moreover, APE2 is required for the recruitment of key checkpoint proteins including ATR, ATRIP, and the 9-1-1 complex onto RPA-ssDNA. Additionally, APE2 associates with Chk1 and brings Chk1 to activated ATR for phosphorylation [86] (Fig. 7). Further investigations are needed to test whether other BER proteins play direct roles in the activation of ATR-Chk1 pathway in oxidative stress.

NER proteins regulate DDR pathways

In the NER pathway, XPA, but not CSB, is required for UV-induced Chk1 phosphorylation at Serine 317 and p53

phosphorylation at Serine 15, suggesting that GG-NER but not TC-NER (transcription-coupled NER) is required for ATR-Chk1 checkpoint signaling [200]. UV-induced phosphorylation of H2AX, ATM, and NBS1 was observed in XPB-deficient cells, suggesting TC-NER may be dispensable for ATM checkpoint signaling [201]. DDB2 and XPC recruit ATM and ATR to UV-damaged sites and promote ATM-Chk2 and ATR-Chk1 checkpoint signaling. Moreover, UV-induced H2AX phosphorylation at Serine 139 in G_1 cells requires both XPA and XPC [202]. In contrast, ATM and ATR seem to be not essential for the recruitment of DDB2 and XPC to UV-damage sites and the NER repair of CPD and 6–4PP [203]. In addition, XPC and XPG are essential for the recruitment of ATM to damage sites after cisplatin treatment, preventing cisplatin-induced apoptosis [204]. Mechanistic studies revealed that Exo1 localizes with XPA to damage sites after local UV irradiation in non-replicating cells and converts NER intermediates into ssDNA to promote DDR signaling [99, 205]. Notably, it was shown in a biochemically defined system that the gap enlargement by Exo1 is essential for ATR-Chk1 checkpoint activation, indicating that NER and ATR signaling pathways are functionally coupled by Exo1 [206] (Figs. 4, 7).

MMR proteins regulate DDR pathways

Cells with MMR defects are more resistant to death by DNA damaging reagents (e.g., alkylating or methylating agents), suggesting the MMR system may play an upstream role for the DDR signaling pathways [207]. Both in vitro and in vivo evidence have shown that MSH2 binds to Chk2 and that MLH1 associates with ATM. The interactions between MMR and DDR proteins (i.e., MSH2-Chk2 and MLH1-ATM) promote the recruitment of DDR proteins to IR-damaged sites and facilitate Chk2 phosphorylation by ATM [196] (Fig. 5). Furthermore, MSH2 is also required for the appropriate relocalization of the MRN complex (Mre11 and Rad50) to IR-induced damage sites [208]. These observations suggest that the MMR pathway plays an essential regulatory role in the ATM checkpoint pathway including ATM, Chk2, and the MRN complex.

S_N1-type DNA alkylating agents induce DNA adducts, such as O6-meG (O6-methylguanine), and activate the ATR-Chk1 pathway. MutSα specifically recognizes O6-meG:T mismatch, but not O6-meG:C, suggesting that MMR proteins can act as direct sensors of mismatches with methylation damage. MutSα also recruits ATR-ATRIP to O6-meG:T, indicating MutSα plays an upstream role for ATR checkpoint signaling [197]. This observation is consistent with the scenario of the anticancer drug 5-fluoro-2′-deoxyuridine (FdU), which may cause mismatch pairs of FdU:G via direct incorporation during DNA replication. ATR-Chk1-dependent DDR is activated by FdU treatment during S phase in a MutSα- and MutLα-dependent manner [209]. Furthermore, MutSα recognizes and associates with FdU:G mismatch pairs in an in vitro binding experiment, whereas FdU may be excised by the uracil glycosylase Smug1 to protect cells from death [210, 211]. These findings suggest that MMR proteins may function as sensors of mismatched DNA lesions and bring DDR proteins to damage sites to activate an appropriate ATR-Chk1 checkpoint.

In response to DNA damage induced by the DNA methylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), MSH2 associates with ATR/ATRIP and regulates MNNG-induced phosphorylation of Chk1 at Serine 317 and SMC1 at Serine 966 [212]. Furthermore, MLH1 is also required for MNNG-induced ATR checkpoint activation such as Chk1 phosphorylation and the colocalization of γ-H2AX and RPA [213]. Furthermore, the MNNG-induced recruitment of ATR, TopBP1, and Chk1 to chromatin requires MutSα and MutLα, suggesting the MMR pathway is required for ATR-Chk1 checkpoint activation [214]. Systematic analysis of the interactions between MMR and ATR-Chk1 pathways via nuclear co-immunoprecipitation assays have shown that MutSα associates with ATR, TopBP1, Claspin, and Chk1 (but not Rad17, Rad9, or RPA), whereas MutLα interacts only with TopBP1 and Claspin [214]. Additionally, the chemotherapeutic drug cisplatin induces MSH2-mediated and ATR-dependent p53 phosphorylation at Serine 15 and Chk2 phosphorylation at Threonine 68 in rat kidney proximal tubular cells. This suggests the involvement of a MMR protein in ATR activation in response to cisplatin-induced oxidative stress [215, 216]. Moreover, MSH2 recruits ATR to damage sites induced by another DNA methylating agent *N*-methyl-*N*-nitrosourea, which is independent of the 9-1-1 complex, Rad17, and RPA [217]. Together, these findings advocate that MMR proteins regulate the ATR-mediated DDR pathway in response to a wide spectrum of DNA damage, including oxidative DNA damage.

HR proteins regulate DDR pathways

The MRN complex is required for the activation of ATM and ATR-dependent DDR pathways in response to DSBs and stalled DNA replication forks, respectively [57, 125, 218]. The MRN complex, MDC1, and H2AX are essential for the recruitment of ATM to the site of DSBs for efficient ATM-mediated DDR activation. However, the prolonged binding to chromatin by repair proteins, such as Mre11, Nbs1 and MDC1, can elicit an ATM-dependent DDR in the absence of DNA damage, suggesting that DDR activation requires the stable association of DNA repair proteins in HR, but not DNA damage *per se* [219]. Furthermore, DSB end processing in HR also contributes to DDR pathways. CtIP is required for DSB end resection, the recruitment of

RPA and ATR to DSBs, and subsequent ATR activation [98, 220]. DNA nucleases (such as DNA2 and Exo1) also contribute to the DSB end resection and subsequent ATM-ATR transition [221] (Fig. 7). Thus, in response to DSBs, ATM may be recruited to the DSB end by the MRN complex and other proteins for activation, whereas ATR is activated after DSB end resection by enzymes such as CtIP and Exo1 (Fig. 7).

Concluding remarks

To maintain genome stability, DNA repair and DDR pathways have evolved as the two major cellular responses to oxidative stress-induced DNA damage [7, 31] (Figs. 1, 7). Dysfunctions in these pathways are linked to cancer and neurodegenerative diseases [6, 33–38] (Fig. 2). Moreover, factors involved in DNA repair and DDR pathways have become therapeutic targets and are currently being tested in both laboratory and clinical studies [151, 222]. We hope we have provided a comprehensive review of the functional interplay between ATM/ATR-mediated DDR pathways and various DNA repair pathways (BER, NER, MMR, and HR) in response to oxidative stress with a focus on higher eukaryotic model organisms.

However, there are still unresolved questions regarding the cellular responses to oxidative DNA damage. Many of the critical barriers in the field include: (1) it is challenging to quantitatively measure oxidatively-generated DNA damage while not measuring intermediate repair products; (2) it remains elusive how accumulation of unrepaired oxidative DNA damage leads to cancer and neurodegenerative diseases; (3) more intense investigations are needed to better understand the potential interplay between ATM/ATR-mediated DDR pathways and DNA damage tolerance pathways in oxidative stress responses; and (4) it is unclear how the ATM-Chk2 and ATR-Chk1 pathways crosstalk with each other in response to oxidative stress. Overall, more intense molecular mechanistic studies of how oxidative DNA damage is repaired and signaled via various integrated DNA repair and DDR pathways will provide new avenues for the treatment of diseases such as cancer and neurodegenerative disorders.

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