Chapter 6
DNA Damage: Cellular Responses, Repair, and Cancer Treatment

Brian M. Cartwright, Phillip R. Musich, and Yue Zou

Abstract The maintenance of genomic stability in the face of endogenous and exogenous sources of DNA damage requires a robust and comprehensive cellular response. This response, appropriately deemed the DNA damage response (DDR), facilitates changes in the cellular environment promoting and coordinating cell cycle arrest, DNA repair, and cell death in cases of extreme or prolonged genomic insult. Initiation of DDR is primarily elicited by three members of the PIKK (phosphatidylinositol-3-kinase-like kinase) family: ATM (ataxia-telangiectasia mutated), ATR (ataxia-telangiectasia and Rad3-related), and DNA-PK (DNA-dependent protein kinase). While all three are required for proper genomic maintenance, DNA-PK lacks the capacity to elicit many of the effects induced by ATM or ATR. For this reason, DNA damage signaling (DDS) generally is considered to occur mainly through ATM and ATR. Recent studies, however, have implicated that DNA-PK can regulate DDS through hindrance of ATM-DDS, giving rise to an evolving view in which all three PIKK family members are essential for regulation of DDS, but not its initiation. This chapter presents a discussion of the signaling within human systems induced by DNA damage as well as an overview of the roles of DDS in promoting DDR-mediated cell cycle arrest, DNA damage repair, and changes to other cellular processes. Within this context, the roles of DDR in current and proposed chemotherapeutics will be explored.

Keywords DNA Damage • DNA Damage Response • Cell Cycle Regulation • Cancer • Chemotherapeutics

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6.1 Introduction

Every day cells are faced with a barrage of genomic insults from endogenous and exogenous sources. Inevitably, these insults lead to DNA damage which must be repaired to maintain genomic integrity. Cells respond to DNA damage by initiating various signaling events. This marks the beginning of the DNA damage response (DDR), and these events lead to activation of cellular pathways ranging from cell cycle arrest, DNA repair, and even cell death in cases of excessive damage. Because of these diverse signaling events, the DDR can regulate cellular fate to promote genomic fidelity on multiple levels. The importance of the DDR is further highlighted by the fact that individuals deficient in DDR function are typically cancer-prone as well as exhibit a wide variety of other pathological complications [1–4].

The DDR is primarily initiated by three PIKK (phosphatidylinositol-3-kinase-like kinase) family members: ATM (ataxia-telangiectasia mutated), ATR (ataxia-telangiectasia and Rad3-related), and DNA-PK (DNA-dependent protein kinase). ATM, ATR, and DNA-PK are serine/threonine kinases that maintain genomic integrity through direct regulation of DDR. While all three kinases are required for proper genomic maintenance, DNA-PK is typically seen as dispensable for overall DNA damage signaling (DDS) [3]. Recently, however, studies have challenged this notion with findings that DNA-PK can regulate ATM activity through direct phosphorylation of ATM as well as potentially mediate cell cycle arrest through Aurora B [5, 6].

In the following sections, the roles of ATM, ATR, and DNA-PK in response to DNA damage will be addressed. First will be a discussion of the roles of the major DDR pathways and PIKK family members in regulation of cell cycle arrest and DNA repair. Then, after briefly reviewing other DNA damage-induced responses, the potential role of chemotherapeutics in eliciting or modulating DDS and the DDR will be considered.

6.2 Maintaining Genomic Fidelity: Cell Cycle Control and DNA Repair

After cells experience DNA damage, there is a complex interplay of signaling pathways. These pathways, mainly orchestrated through ATM and ATR, lead to a fine-tuned response which alters cell cycle progression, nucleotide metabolism, and other parameters involved in promoting an optimal environment for maintaining DNA integrity [2, 4]. The following sections address cell cycle checkpoint activation and control, various types of DNA repair, and the multifaceted interplay through which checkpoint activation modulates the DDR.

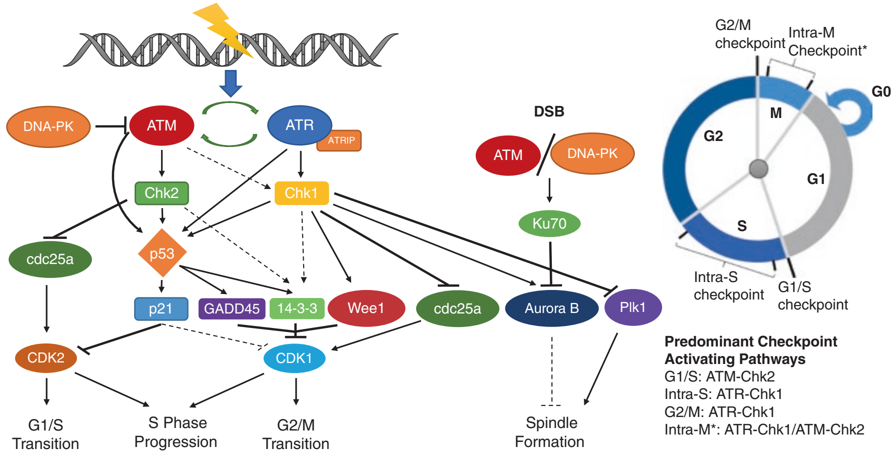

Fig. 6.1 Cell cycle regulation by the DNA damage checkpoints. DDS through ATM, ATR, and DNA-PK control the cell cycle at multiple levels. ATM and ATR are the primary regulators of cell cycle arrest through their downstream substrates, kinases Chk2 and Chk1 respectfully. Chk1 and Chk2 phosphorylate multiple targets resulting in either their direct inhibition, degradation, or activation of negative regulatory function. DNA-PK is thought to play a lesser role in DDS, though it has been shown to regulate ATM activity as well as potentially be involved in spindle formation checkpoint (*intra-M checkpoint). DNA repair pathway engagement is cell cycle-dependent. Where BER, NER, and NHEJ occur in all phases of the cell cycle, HR and ICLR only occur during S and G2 phases

6.2.1 Cell Cycle: Checkpoint Control and Arrest

When cycling cells incur DNA damage, they activate processes to arrest cell cycle progression. This arrest prevents the accumulation of mutations in both themselves as well as potential daughter cells. While the exact regulation of cell cycle progression is out of the scope of this review, it is important to note that DDS mediated by both ATM and ATR regulate the cell cycle through downstream modulation of cell cycle progression factors (Fig. 6.1).

ATM and ATR function to regulate the cell cycle in response to different stresses and largely during different phases of the cell cycle. ATM and its effector kinase Chk2 (checkpoint kinase 2) respond mainly to DNA double-strand breaks (DSBs). While ATM is active throughout the cell cycle, it plays a predominant role during the G1 phase of the cycle and into the G1/S transition. During this phase, the cells are preparing to synthesize new DNA, and damage occurring during G1 leads to activation of ATM through autophosphorylation on its Ser1981 residue which leads to the dissociation of the ATM dimer to monomer and further phosphorylation of ATM at Ser367, Ser1983, and Ser2996 [7, 8]. ATM then phosphorylates Chk2 on Thr68, leading to its activation and priming the cell cycle arrest cascade [3, 9]. Chk2 has a myriad of substrates; however, two are of primary interest regarding cell cycle arrest: cdc25A and p53. Chk2 phosphorylates the protein phosphatase cdc25A on Ser123, limiting its activity as well as targeting it for ubiquitination and subsequent

proteasomal degradation. Restriction of cdc25A activity prevents the removal of the inhibitory phosphoryl groups attached to Thr14 and Tyr15 of CDK2 (cyclin-dependent kinase 2). CDK2, when complexed with cyclin E, is required for the progression from the G1 to the S phase of the cell cycle. Therefore, reduction of cdc25A activity facilitates arrest of the G1/S transition due to decreased activation of CDK2 [9, 10]. In addition to down regulation of CDK2 through inactivation and removal of cdc25A, ATM and Chk2 coordinate the induction of $p21^{WAF1/Cip1}$ which directly inhibits CDK2 through interaction via its N-terminal Cy1 motif [11]. This induction is part of a three-step mechanism. The first step is the phosphorylation of p53 on Ser20 by Chk2, leading to a conformational shift in p53 allowing for its dissociation from its normal sequestering protein MDM2 (mouse double-minute 2 homolog), an E3 ubiquitin ligase. This dissociation allows the second step to occur in which p53 is further phosphorylated by ATM at Ser15. Modification of this site increases the transcriptional activity of p53 resulting in the induction of $p21^{WAF1/Cip1}$. The third step involves ATM phosphorylation of MDM2 on Ser395, serving as a backup mechanism to prevent its rebinding to p53 and, thus, ensuring proper p53 activation and induction of increased transcription of DDR protein genes [9, 12].

While the G1/S checkpoint is initiated through ATM, ATR and its effector kinase Chk1 (checkpoint kinase 1) are the primary activators of the intra-S (primarily in response to DNA damage-induced replicative stresses) and G2/M checkpoints. Longer single-strand DNA (ssDNA) generated through replicative stress or DSB end resection is rapidly coated with replication protein A (RPA), the RPA-ssDNA complex recruits ATR-ATRIP (ATR-interacting protein) as well as Rad17 and the 9–1-1 (Rad9-Rad1-Hus1) complex. Binding of the 9–1-1 complex to RPA is a signal for TopBP1 (topoisomerase-binding protein 1) recruitment [13–16]. TopBP1 is an allosteric regulator of ATR, promoting ATR activation through its autophosphorylation on Thr1989 [17–19]. After complexing with claspin, activated ATR then phosphorylates Chk1 on Ser317 and Ser345 to elicit intra-S and G2/M checkpoint activation and arrest of cell cycle progression [20]. Like Chk2, Chk1 also phosphorylates cdc25A on Ser123, leading to its inhibition and targeted ubiquitin-mediated degradation. While this action promotes the G1/S checkpoint, it also serves as a safeguard should cells be able to complete DNA repair and reenter the cell cycle. Additionally, this functions to facilitate the intra-S checkpoint through the inability of phosphorylated CDK2 to form an active CDK2/cyclin A complex, resulting in premature stalling or termination of DNA synthesis [20, 21]. The intra-S checkpoint is mediated further by parallel phosphorylation of p53 by ATR and the subsequent gene induction cascade as presented previously through p53 with respect to ATM and Chk2.

In addition to $p21^{WAF1/Cip1}$ induction, p53 also promotes the transcriptional upregulation of GADD45 and 14–3-3 which both regulate the G2/M checkpoint upon ATM and ATR activation. GADD45, commonly known as GADD45a, functions to directly bind to and suppress CDK1 (cyclin-dependent kinase 1) while it is in complex with cyclin B1. In this way, binding of GADD45 to the CDK1/cyclin B1 complex inhibits CDK1 activity and the transition from G2 into mitosis. Of importance also is the fact that GADD45 has no effect on the activity of the CDK1/cyclin E complex which is active during G1 further tailoring GADD45 as a G2/M checkpoint inducer [22]. The

active forms of both Chk1and Chk2 phosphorylate cdc25C at Ser216 priming it for binding by the 14–3-3 complex which leads to the nuclear export and cytosolic sequestration of cdc25C. This sequestration is necessary for G2/M checkpoint arrest because cdc25C, a protein phosphatase similar to cdc25A, functions to remove inhibitory phosphorylations from CDK1. Unphosphorylated CDK1, complexed with cyclin B1, serves as the regulatory kinase for cell cycle progression from G2 into mitosis. Therefore, like with CDK2, inhibition of the activating phosphatase leads to an increased accumulation of inactive phosphorylated CDK1 [23]. These mechanisms, however, are not alone in the regulation of the G2/M checkpoint; Chk1 also activates Wee1, a kinase that phosphorylates CDK1 on Thr14 and Tyr15. This phosphorylation inhibits CDK1 activity. These phosphorylations by Wee1 enhance the inhibition of CDK1 brought about by the cytoplasmic sequestration of the protein phosphatase cdc25C [24]. To further promote cell cycle arrest, downstream kinases activated by ATM and ATR lead to phosphorylation of Plk1 (Polo kinase 1), targeting it for degradation. The degradation of Plk1 promotes a prolonged and robust G2/M arrest by Wee1 as under normal physiological conditions Plk1 phosphorylates Wee1, leading to its degradation and allowing for a normal G2/M transition [25].

Lastly, the intra-M, or mitotic spindle checkpoint, serves as a last line of defense in protecting genomic integrity following DNA damage. Unlike the other checkpoints, all three DDR PIKK family members are involved in the initiation of the intra-M checkpoint. ATR functions through Chk1 to activate the Aurora B kinase which subsequently delays abscission and progression through cytokinesis [26]. Additionally, Chk1 phosphorylates Plk1 preventing its active role in promoting centrosome formation and mitotic spindle assembly [25]. ATM and DNA-PK are implicated in the intra-M checkpoint via regulation of Ku70 phosphorylation at Ser155 [5]. While it is unknown if this phosphorylation is dependent on ATM or DNA-PK, this phosphorylation event is of importance as it leads to the interaction of Ku70 with Aurora B, inhibiting the latter's kinase activity [5].

It is important to note that activation of ATR and ATM are both likely to invoke the DDR and cell cycle arrest regardless of cell cycle phase due to known cross activation and regulation between the DDS pathways [27–30]. In addition, recent reports have shown that DNA-PK is capable of directly modulating ATM activation through inhibitory phosphorylation of ATM, leading to reduced DDS through ATM following DNA damage. Because of this, DNA-PK could potentially regulate ATM induction of checkpoints and subsequent cell cycle arrest [6]. This implicates an even more complex regulation of DDR than previously described.

6.2.2 DNA Damage Repair

Upon sensing of DNA damage, cells activate repair processes to restore genomic integrity. Multiple DNA repair mechanisms have evolved to defend genomic integrity against a variety of different endogenous and exogenous sources of DNA damage (Fig. 6.2). Specific repair pathways engage depending on the type of lesions

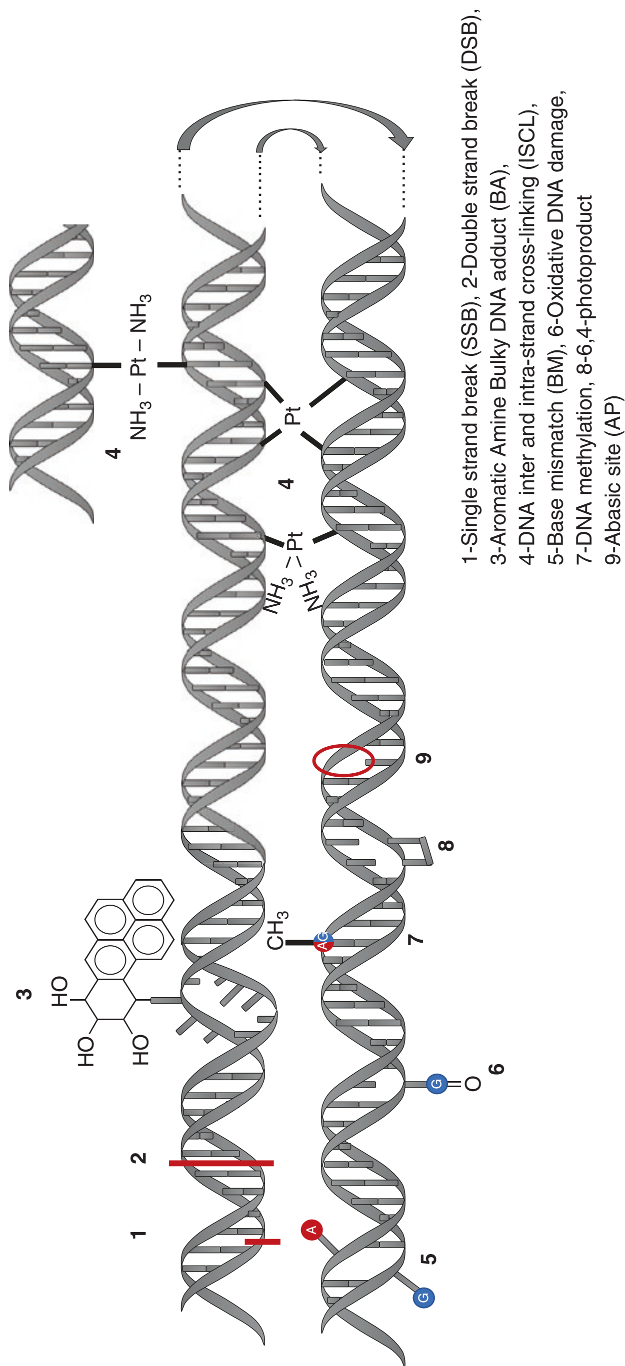

Fig. 6.2 Common types of DNA damage

Table 6.1 Types of DNA repair and activating factors

DNA repair pathway	Types of damage
BER	Alkylation, spontaneous depurination or depyrimidination, deamination, oxidation, single-strand breaks
NER	Bulky adducts (benzo(*a*)pyrene, photoproducts, etc.), intrastrand cross-links
MMR	A > G mismatches, T > C mismatches, trinucleotide expansions, base deletions
NHEJ/Alt-NHEJ	Double-strand breaks
HR	Double-strand breaks
ICLR	Inter- and intrastrand cross-links

present (Table 6.1). The following is a summary of major pathways as well as the DDS effects that influence them.

6.2.2.1 Base Excision Repair (BER)

The most commonly occurring lesions in DNA are those arising from oxidation, alkylation, or spontaneous depurination/depyrimidination (abasic site formation). These lesions trigger the base excision repair (BER) process facilitating the removal of the damaged base and processing of the newly generated abasic site (Fig. 6.3a). The initial step in BER is hydrolysis of the N-glycosylic bond of the modified base by various DNA glycosylases leading to the formation of an apurinic/apyrimidinic (AP) site. The AP site then is modified by poly (ADP-ribose) polymerase 1 (PARP1) through a process known as PARylation. PARylation is a process in which a polymer of ADP-ribose (PAR) is added to DNA or proteins through consumption of NAD^+ [1, 31]. This PARylation event generates the primary signal of DDS in BER as PARylation of DNA, as well as auto-PARylation of PARP1, leads to the recruitment of several downstream proteins associated with BER including XRCC1 (X-ray repair cross-complementing protein 1), OGG1 (8-oxoguanine glycosylase 1), and others. This recruitment is based on binding to PAR chains facilitated through various binding motifs [31].

In addition, previous reports have shown that protein deacetylases can influence DDS signaling of BER through the initial substrate specificity of glycosylases [32]. Examples include the effects of SIRT1 on TDG (thymine DNA glycosylase) and APE1 (AP-endonuclease 1). SIRT1 deacetylation of TDG changes the substrate specificity of TDG, whereas deacetylation of APE1 promotes binding with XRCC1. In the case of TDG, the deacetylation by SIRT1 promotes excision of the nucleoside analogue 5-fluorouracil (5-FU), whereas unacetylated TDG mainly targets methylated substrates [33, 34]. Loss of SIRT1 deacetylation has the potential of muting the DDR by preventing the recognition of the lesion and the generation of the AP site. The same is true of APE1 whose deacetylation at Lys6 and Lys7 by SIRT1 leads to interaction with XRCC1. This

APE1-XRCC1 interaction promotes the glycosylase activity of APE1, enhancing AP site generation [35].

Once PARP1 modifies the AP site, other repair proteins are recruited to finish processing the AP site. WRN (RecQ or Werner protein), a helicase and exonuclease, is recruited to stimulate polymerase β (POLβ) binding for insertion of the missing nucleotide or nucleotides. DNA ligase III (LIGIII) then ligates the DNA strand [36]. This process is DDS-independent; however, it has been shown that DDS-dependent deacetylation of WRN by SIRT1 promotes its exonuclease activity in cases of long-patch BER. In this way, signaling by SIRT1 functions to improve BER endonuclease activity which leads to the removal of up to ten nucleotides [37]. Under long-patch BER, polymerase δ (POLδ) or polymerase ε (POLε) catalyzes the repair DNA synthesis; FEN1 (flap endonuclease I) then removes the displaced DNA strand. The DNA strands are then ligated by DNA ligase I (LIGI) [32].

6.2.2.2 Nucleotide Excision Repair (NER)

Bulky DNA lesions, such as those caused by UV-induced photoproducts, polycyclic aromatic hydrocarbons, and cross-linking chemotherapeutics (e.g., *cisplatin*), can distort the DNA helical structure. Nucleotide excision repair (NER) repairs these types of lesions (Fig. 6.3b). NER pathways come in two distinct forms: global genome repair (GG-NER) and transcription-coupled repair (TC-NER) [38–40].

In human GG-NER, initial recognition of DNA damage is done by XPC (XP complementation group C)-HR23B or together with DDB1-DDB2/XPE [damage-specific DNA-binding protein 1 or 2- xeroderma pigmentosum complementation group E (XPE)] upon UV irradiation. While the XPC-HR23B complex can localize to damaged DNA by itself, the efficiency of recruitment is enhanced following polyubiquitination by the DDB1-DDB2-CUL4A/B complex. This polyubiquitination assists XPC in binding at the DNA lesion [41]. Once XPC binds to a bulky DNA adduct, transcription factor IIH (TFIIH) is recruited. Two components of the TFIIH, XPB and XPD (XP complementation groups B and D, respectively), mediate strand unwinding. The single-strand DNA (ssDNA) generated is rapidly bound by RPA (replication protein A), and XPA (XP complementation group A) is recruited for verification of the DNA damage. XPA also appears to stabilize the repair intermediate and serves to recruit the endonucleases XPG (XP complementation group G) and XPF-ERCC1 (excision repair cross-complementation group 1) [42, 43]. XPG facilitates the 3′ incision, while XPF-ERCC1 does the 5′ incision [44, 45]. Then, the adducted DNA fragment of 22–30 nucleotides is removed, followed by recruitment of PCNA (proliferating cell nuclear antigen) by RFC (replication factor C). PCNA loads one of three DNA polymerases (delta, epsilon, or kappa) onto the DNA facilitating its repair synthesis. DNA ligase I or the ligase III-XRCC1 complex then seals the DNA termini [38]. TC-NER follows a similar series of steps with the exception that ERCC6/CSB (excision repair cross-complementing group 6, Cockayne syndrome B) performs the initial damage recognition, not XPC-

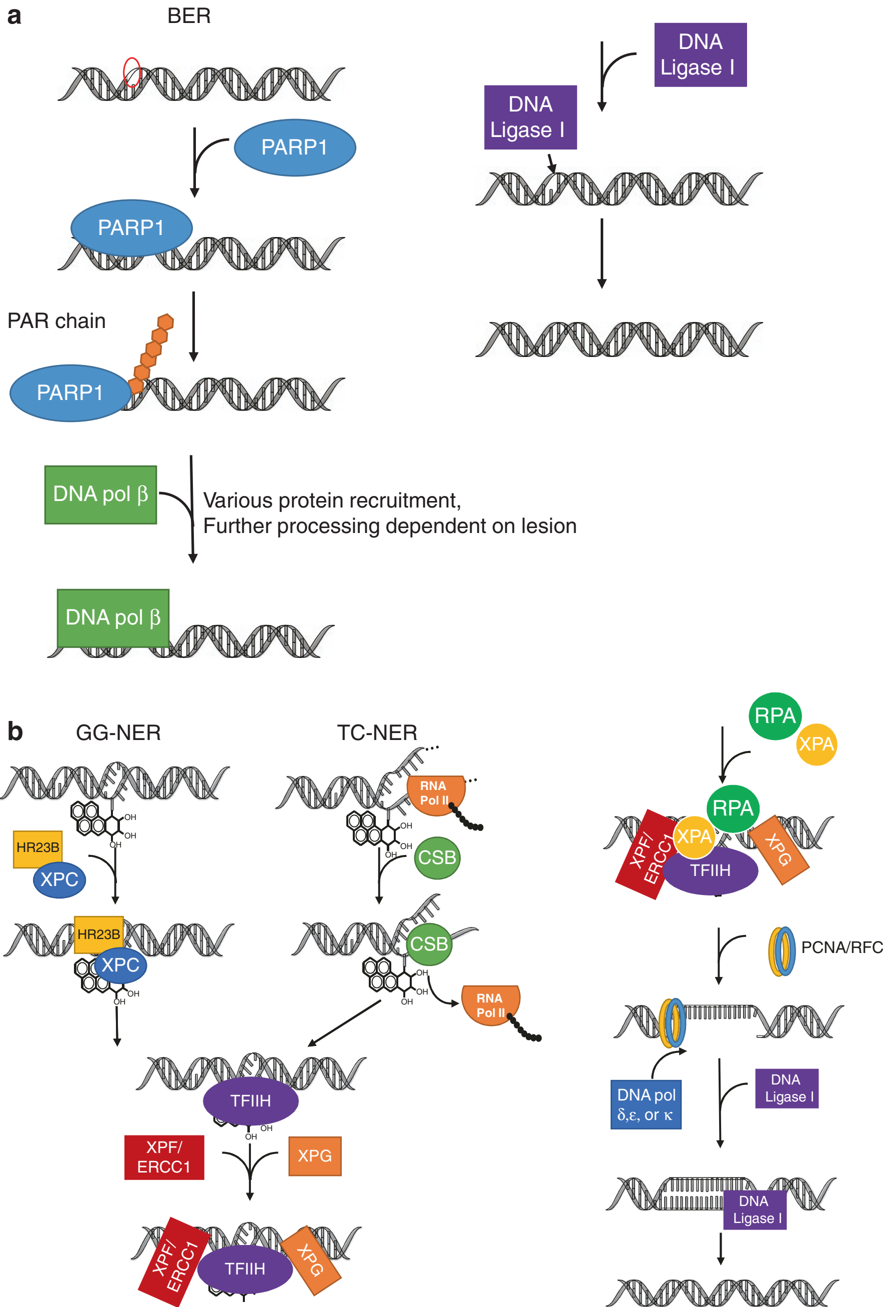

Fig. 6.3 Selected DNA damage repair pathways. DNA damage must be repaired to prevent mutations from occurring within cells. (**a**–**d**) summarize some of the most common forms of DNA damage repair. (**a**) Base excision repair (BER) [short patch of an apurinic site], (**b**) nucleotide excision repair (NER) of a bulky aromatic adduct, (**c**) nonhomologous end joining (c-NHEJ and alt-NHEJ), (**d**) homologous recombination (HR)

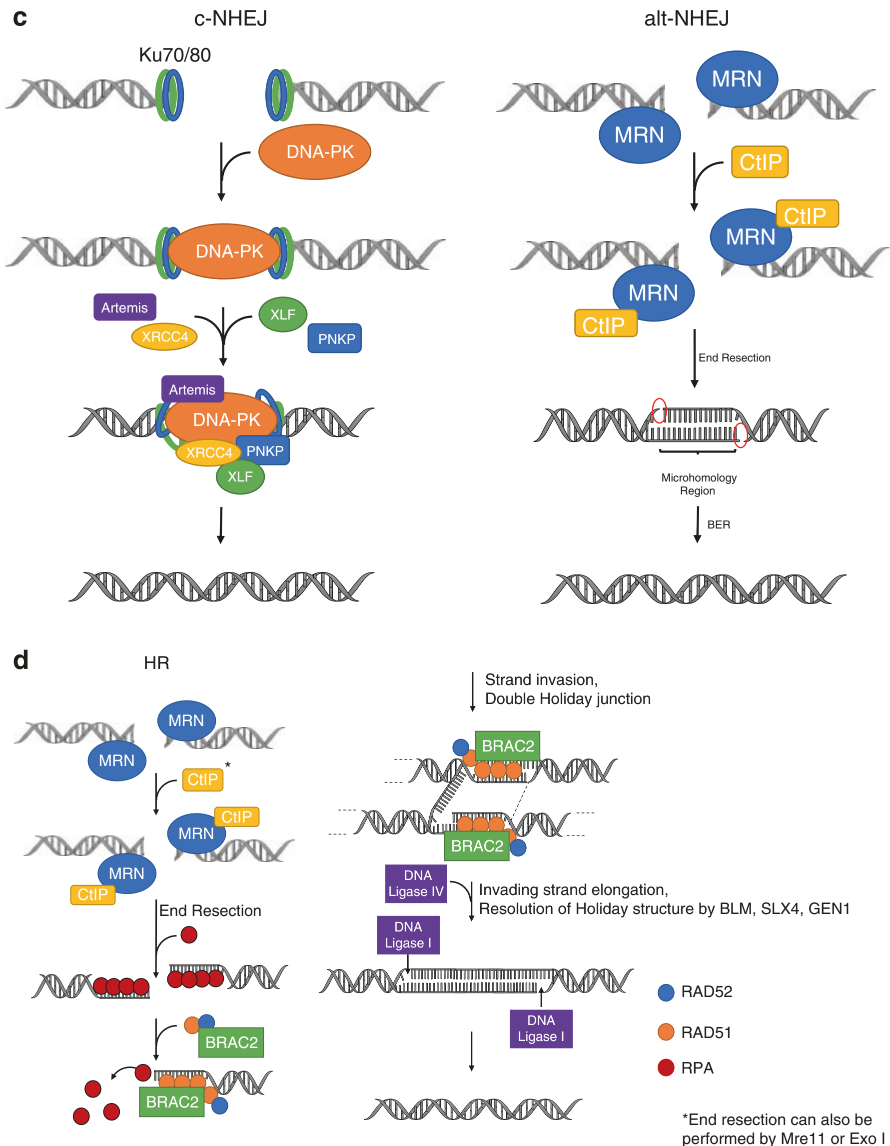

Fig. 6.3 (continued)

HR23B, and is followed by the removal of stalled RNA polymerase II from the DNA lesion before repair proceeds. This is done through ubiquitin-mediated removal and subsequent degradation that is dependent on ERCC6/CSB [46].

In addition, some other key events are involved in NER. For example, SIRT1 deacetylation of XPA promotes the interaction of XPA with other NER factors. This serves to increase recruitment and activity of XPG and ERCC1-XPF [47].

Additionally, ATR-mediated XPA phosphorylation enhances XPA stability by inhibiting HERC2-medated ubiquitination and subsequent degradation [48].

6.2.2.3 Mismatch Repair (MMR)

Mismatching of bases typically occurs during replication or after the deamination of cytosine in DNA. In brief, either MutSα (MSH2-MSH6 heterodimer) or MutSβ (MSH2-MSH3 heterodimer) binds to the mispaired bases. MutSα preferentially recognizes post-replicative mispairings as well as methylated bases, whereas MutSβ recognizes insertion repeats or deletion loops [49, 50]. Both versions of MutS then serve as a scaffold signaling the recruitment of various factors to excise and replace the mismatched or damaged DNA. Active HDAC 6 and 10 decrease the stability of MSH2 and enhance its degradation, indicating that acetylation of MSH2 is required for scaffold stability and MMR [32].

6.2.2.4 Nonhomologous End Joining (NHEJ)

DSBs are repaired by two major pathways, the nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ corrects strands brakeage through direct modification and ligation of broken strands without regard for sequence homology. To date, two distinct forms of NHEJ have been discovered: classic-NHEJ (c-NHEJ) and alternative-NHEJ (alt-NHEJ) (Fig. 6.3c) [51].

c-NHEJ initiates upon binding of Ku70/Ku80 heterodimer to the termini of DSBs. This leads to the recruitment of DNA-PKs to the Ku70/Ku80-bound DNA. Upon DNA binding, DNA-PK undergoes activation and autophosphorylation at multiple sites in both its ABCDE (T2609, S2612, T2620, S2624, T2638, and T2647) and PQR (S2023, S2029, S2041, S2051, S2053, and S2056) domains [52]. These events promote stability of the DNA-PK/DNA complex and facilitate end interaction while recruiting other factors such as Artemis, XRCC4, XLF (XRCC4-like factor), PNKP (polynucleotide kinase/phosphatase), and DNA ligase IV [53]. If the broken DNA ends are not compatible for direct ligation by DNA ligase IV, they are first processed. The Artemis nuclease is responsible for end resection during this process. PNKP then modifies the ends of the DNA to be recognizable substrates for DNA ligase IV by either removing or adding phosphoryl groups from the 3′ and 5′ termini, respectively. While DNA-PK phosphorylates all of the proteins it recruits, currently there is no evidence that any of these individual phosphorylation events are necessary for NHEJ [54].

An early event following DNA-PK activation is the phosphorylation of histone variant H2AX at Ser139; this phosphorylated form is known as γH2AX. This is an important step as γH2AX serves to amplify the signal of the DSB and aids in the recruitment of many factors involved in repairing the breakage by promoting chromatin reorganization. One of the factors recruited is ATM which modulates NHEJ to either promote its efficiency or to promote a shift to HR [30, 55]. The molecular

processes governing pathway decision for DSB repair are presented in the homologous recombination (HR) section (Sect. 2.2.5).

There are many factors which modulate c-NHEJ efficiency. PARylation by PARP1 directly stimulates DNA-PK activity. The PARP1 binding to the DNA-PK/Ku complex elicits a structural change in the complex, facilitating more efficient repair [31]. PARylation of DSB termini also increases early recruitment of DNA ligase IV through a scaffolding event caused by interaction between the BRCT domain of DNA ligase IV and the PAR chains. This has the potential to promote a more efficient repair due to decreased lag time in recruitment of DNA ligase IV [56]. Deacetylation also plays a distinct role in NHEJ. KAP1 (KRAB-associated protein-1) is deacetylated by SIRT1 which promotes chromatin relaxation and invasion of NHEJ repair factors. SIRT1, as well as HDAC 1–3, deacetylates Ku70, promoting its binding to DSB termini, and subsequently increases NHEJ efficiency. Lastly, SIRT6 promotes DNA-PK localization to DSBs as well as DNA-PK/Ku complex stability [32].

Alt-NHEJ is independent of both DNA-PK and Ku70/80. In alt-NHEJ, PARP1 recognizes DSBs which have already undergone end resection and have complementary microhomology regions (1–10 nucleotides) which have annealed. PARP1 then PARylates the termini of the breaks, signaling for DNA ligase III/XRCC1 recruitment and ligation of what now appear as SSBs. Alt-NHEJ is more error prone than c-NHEJ as it requires formation of microhomologies and greater amounts of DNA may be resected prior to annealing to facilitate microhomology formation [31, 57].

6.2.2.5 Homologous Recombination (HR)

Homologous recombination is imperative for the maintenance of genomic stability during development and preservation of stem cell populations. Active in S and G2 phases of the cell cycle, HR (Fig. 6.3d) requires both ATM and ATR kinases to function.

In HR, the MRN complex, made up of Mre11, Rad50, and Nbs1, recognizes DSBs leading to the recruitment of ATM. Once bound to the MRN complex, ATM undergoes activation through autophosphorylation at Ser1981. Another early ATM substrate in this process is histone H2AX which is rapidly phosphorylated at Ser139 to form γH2AX, which induces recruitment of MDC1. MDC1 serves to form an adaptor complex with ATM-Nbs1 as well as with γH2AX. The MDC1-ATM-Nbs1 complex amplifies the γH2AX signal through further phosphorylation of H2AX, while the MDC1-ATM-γH2AX complex recruits E3 ubiquitin ligase RNF8. RNF8 ubiquitinylates various histones surrounding the DSB site, serving to loosen the local chromatin structure as well as provide a signal for recruitment RNF168, another E3 ubiquitin ligase, through its ubiquitin-binding domain. RNF8 and RNF168 function through the E2 ubiquitin ligase UBC13 to promote the recruitment and retention of various NHEJ and HR factors: 53BP1, RAD18, BRCA1, BRCA1-A, HERK2, etc. [55, 58].

For homologous recombination to continue, the DSB ends must have one strand resected. One of three exonucleases performs this: Mre11, Exo1, or CtIP [59–61]. The BRAC1/BARD1 complex enhances this resection and facilitates pathway selection through direct displacement of 53BP1 from DSBs as well as through recruitment of various factors required for the end resection through its BRCT motifs. BRAC1 and 53BP1 are both recruited through RNF8 signaling; however, they function in a dynamically opposed manner. While BRAC1 promotes end resection and repair through HR, 53BP1 functions to inhibit end resection and promote repair through NHEJ [58]. So why would BRAC1 and 53BP1 both be recruited by the same initial signaling event? This can be attributed to 53BP1 being necessary for effective ATM activation as well as being required for ATM-mediated checkpoint kinase activity through Chk2 [62]. In this way, both BRAC1 and 53BP1 are required for appropriate HR function if only to allow more time to complete the required repair.

The BRAC1/BARD1 complex promotes the recruitment of Abraxas-RAP80, BRIP1 helicase, and CtIP. BRAC1 forms a complex with each protein partner to form either BRAC1-A, BRAC1-B, or BRAC1-C, respectively. BRAC1-C functions to induce end resection through CtIP's exonuclease activity. The BRAC1-A complex regulates this resection to ensure that ends are not over processed. BRAC1-B removes secondary DNA structures that occur during this process as well as alleviating any occurring before end resection that might be due to cross-linking, replication fork stalls, or replication fork collapses [63, 64].

The ssDNA generated by end resection is rapidly bound by RPA. Following RPA binding, recombination initiates with the Rad52-mediated loading of Rad51 recombinase onto the ssDNA of resected DSB. Rad51 displaces RPA from ssDNA and is dependent on the BRAC2-PALB2 complex which functions to localize BRAC2 and Rad51 to the ssDNA and allows for efficient Rad51 loading by BRAC2 [64, 65]. Rad51 plus other HR proteins forms the nucleoprotein filament that is responsible for sister chromatid invasion. During this process, Rad51 mediates strand displacement and invasion of the 3′ end of that strand into the sister chromatid, resulting in the formation of a D-loop structure with the invading strand base-paired to the intact complementary strand. DNA synthesis then extends the invading strand resulting in Holliday junction formation. Once this junction is formed, the other 3′ strand of the DSB enters the complex leading to formation of a double Holliday junction where this strand then is elongated. The resolution of these structures is still not well understood in eukaryotic systems, but several proteins such as BLM, MUSLX4, or GEN1 could be implicated due to their helicase and nuclease activities [66–68].

In addition to the responses presented above, there are other measures which help to ensure pathway selection and successful completion of HR. One example of this is deacetylation of CtIP by SIRT6 which promotes its function in end resection. SIRT6 depletion decreases the amount of RPA recruited to resected DSBs implicating its importance in supporting HR [69]. This contrasts with its function in supporting NHEJ, as presented previously, and likely has cell cycle dependence, as HR is only available during S and G2 phases.

6.2.2.6 Interstrand Crosslink Repair (ICLR)

Some lesions are highly complex and require multiple pathways to repair them efficiently. The repair of interstrand cross-links requires activation of NER and/or HR in combination with the Fanconi anemia (FA) pathway. The combined process, known as interstrand cross-link repair (ICLR) for simplicity, is initiated by either XPC or ERCC6/CSB if the cross-link can be removed through NER or by the FA pathway protein FANCM (Fanconi anemia complementation group M) if it is within a stalled replication fork [41, 46, 70]. As NER was previously described (Sect. 2.2), the following will address the role of FA in ICLR.

Initial recognition of an interstrand cross-link at a stalled replication fork is performed by FANCM which is part of the Fanconi anemia core complex: FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM. FANCM along with binding partners FAAP24 and MHF1 forms a complex that supports the DEAH-helicase activity of FANCM leading to strand displacement at the branches of a stalled replication fork [71]. This promotes the accumulation of RPA on the displaced ssDNA and subsequent likely recruitment and activation of ATR/ATRIP as is seen in HR [72]. Simultaneously, FANCL induces ubiquitination on FANCD2 a Lys561. This is crucial for FANCD2/FANCI complex localization to the site of DNA damage [73]. FANCD2/FANCI promotes the recruitment of FAN1 and SLX4 (FANCP) which function alongside XPF/ERCC4 (FANCQ) to perform incisions upstream and downstream of the interstrand cross-link, respectively [74]. After the incisions, HR takes over the DNA repair process. It is important to note that when the FA pathway is referenced, there are several alternative names for HR proteins, but otherwise they possess the same function. These are as follows: BRAC2 (FANCD1), PALB2 (FANCN), BRIP1 (FANCJ), and RAD51 (FANCO).

ATR serves a particular role in the FA pathway because its DDS activity is required to ensure ICLR through the main FA complex as well as the FANCD2/FANCI complex. First is the ATR-dependent phosphorylation of FANCM at Ser1025 which is required for FA pathway activity and sufficient G2/M arrest [75]. ATR also activates the FANCD2/FANCI complex through phosphorylation of FANCD2 at Thr691 and Ser717 and FANCI at several SQ/TQ motifs [76, 77]. ATR also phosphorylates FANCA at Ser1449 and promotes phosphorylation of FANCE by Chk1 [78, 79].The result of which in all instances promotes FANCD2 ubiquitination, recruitment to DNA damage sites, and activity [74].

6.2.3 Integrating the Signals: Checkpoint Regulation of DNA Repair

When considering the DDR, it is essential to consider the transiently activated checkpoints as well as its regulation of DNA repair as coordination between the two systems is indispensable for successful maintenance of genomes. Also importantly, the coordination is likely cell cycle-dependent in most cases. While this is based on

the premise that some DNA repair protein expression is limited to certain phases of the cell cycle, this concept has much broader implications when investigating integrated control on pathway selection and repair efficiency [80–82].

The checkpoint control of DNA repair is seen at multiple levels stemming from ATM, ATR, and DNA-PK activity. For instance, ATR regulates NER following UV irradiation through direct binding and phosphorylation of XPA at Ser196 promoting its stability and nuclear import following UV irradiation [83–86]. This process is dependent on PKA phosphorylation of ATR at Ser435, and loss of this site leads to reduced ATR-XPA binding as well as delayed XPA recruitment to sites of DNA damage [87]. This effect is found to occur primarily in the S phase of the cell cycle and to be p53 dependent. This is in contrast to XPA nuclear import during G1 or G2 phases in which XPA nuclear import is p53/ATR independent; in G1, the UV-induced import is muted, while in G2, XPA accumulates in the nucleus regardless of DNA damage [88]. Checkpoint control of NER is further enacted by p53 which is a target of all three apical kinases as well as secondary kinases, Chk1 and Chk2. p53 upregulates gene expression of NER proteins following a variety of genomic insults resulting in increased DDB2, XPC, XPF, and XPG levels [89, 90]. Active p53 is also known to be involved in the recruitment of XPC as well as TFIIH to sites of UV damage where it facilitates improved DNA damage recognition and repair [91, 92].

Checkpoint control of DNA repair also extends to BER, HR, and NHEJ. BER activity is modulated through activated p53's direct binding to three BER enzymes: APE/REF1, OGG1, and DNA polymerase beta. This binding stimulates the recognition, excision, and respective repair activities of these enzymes leading to enhanced BER [93, 94]. ATM, ATR, and DNA-PK all collaborate to promote effective HR through the regulation of the RPA-p53 interaction [30]. The interaction of RPA-p53 typically promotes NHEJ through sequestration of RPA; however, Ser37 and Ser46 phosphorylation of p53 by ATM and ATR, respectively, along with RPA32 phosphorylation by DNA-PK leads to dissociation of the RPA-p53 complex and a switching to HR [30, 95].

In addition to the effects listed previously, direct cycle control of DNA repair occurs through cyclic expression of repair factors and regulation by cyclin dependent kinases. In brief, many proteins involved in DNA repair are only expressed at certain points throughout the cell cycle. For instance, gene-encoding proteins for mismatch repair are almost exclusively expressed in S phase, whereas most genes for ICLR are expressed in S-M phases. For more information regarding cell cycle expression of DNA repair proteins, we would point interested readers to the recent work by Mjelle et al. as the topic is quite expansive [80]. Cyclin-dependent kinases also play a role in promoting DNA repair efficiency and pathway selection. CDK1, the cyclin associated with promoting G2/M transition, is responsible for phosphorylation of CtIP at Ser327 and Thr847 leading to increased BRCA1 binding by CtIP and enhanced end resection favoring HR repair [96, 97]. In contrast, CDK2, which is responsible for promoting the G1/S transition, phosphorylates CtIP at Ser276 and Thr315 leading to its inhibitory isomerization by prolyl isomerase Pin1. This prevents end resection of DSBs in early G1 and early S phases leading to the promotion of NHEJ over HR in these instances [98]. These events represent a minor fraction of the cell cycle control of DNA repair. For more information, we would suggest the recent works by Hustedt and Warmerdam [81, 82].

6.3 Other Cellular Responses to DNA Damage

Cells respond to DNA damage in an assortment of ways in addition to the DDR. Most of these responses are geared at increasing DNA repair efficiency and promoting cell survival. Processes such as autophagy and inflammation serve to facilitate these pro-survival responses; in contrast, there are times where the DNA damage is too extreme to allow for cell survival. While cell death may be viewed as a negative event, under normal conditions it serves to protect the organism from tumorigenesis. In cases of extreme DNA damage, ATM and ATR can initiate pro-apoptotic signaling through the tumor suppressor p53. These responses, and their interplay with processes previously mentioned, are detailed in the following sections.

6.3.1 Autophagy

Autophagy is a catabolic process by which proteins and organelles are degraded to either remove damage or provide usable metabolic constituents in times of stress. Typically, autophagy is induced under cellular damage or starvation; however, it also can be induced by other taxing events such as DNA damage [99]. Several studies have established that the induction of autophagy following DNA damage is cytoprotective and plays an integral role in protecting cells upon DNA damage induced by chemotherapy, radiation, or other sources [100–102].

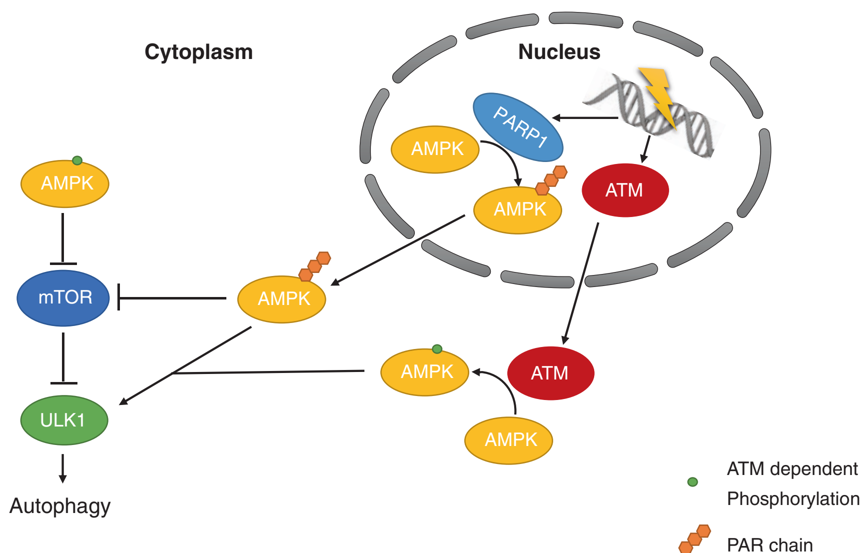

Fig. 6.4 DNA damage regulation of autophagy

The regulation of autophagy by the DDR occurs on multiple levels (Fig. 6.4). The regulation of autophagy by the DDR can occur through ATM which phosphorylates cytoplasmic AMPK (5′ adenosine-monophosphate-activated protein kinase) on Thr172 resulting in its activation. This leads to the induction of autophagy by two mechanisms. The first is inhibition of the kinase mTOR (mammalian target of rapamycin) through activation of TSC1/2 (tuberous sclerosis complex 1 or 2). AMPK phosphorylation of TSC1/2 causes TSC1/2 interaction with mTOR, leading to repression of mTOR activity. Under normal conditions, mTOR inhibits autophagy through an inhibitory phosphorylation at Ser757 of ULK1 (Unc-51-like kinase 1), the kinase responsible for initiating autophagosome formation. The second is direct activation of ULK1 by AMPK through phosphorylation of ULK1 at Ser317 [103–105]. In addition to this function in global autophagy, ATM has recently been shown to mediate mitophagy and pexophagy, two specific types of autophagy targeting mitochondria and peroxisomes, respectively, following exposure to oxidative stress [106, 107]. Mitophagy and pexophagy induction by this mechanism could have implications in lowering cellular ROS (reactive oxygen species) levels following irradiation or other oxidative damaging therapies allowing for cancer cell resistance and survival to therapies of this nature [106, 107].

Another activator of autophagy following DNA damage is PARP1. PARP1, like AMPK, plays a bifunctional role in the activation of autophagy. The first is through its global activity following oxidative DNA damage in which it PARylates both DNA and proteins. This activity leads to the consumption of NAD^+ which eventually leads to a downstream depletion of ATP resulting in AMPK activation. As noted previously, AMPK activation leads to autophagy induction through negative regulation of mTOR through TSC1/2 and through positive regulation of the ULK1 [108]. In addition, PARP1 has recently been shown to be in complex with nuclear AMPK. Under starvation, a cellular state characterized by oxidative stress and DNA damage, nuclear AMPK is PARsylated and subsequently exported into the cytosol [109]. The importance of this event is critical as it allows for early activation of an autophagic response following DNA damage without the need for transcription to occur. Additionally, activation in this fashion does not affect the independent ATM activation of cytoplasmic AMPK [109]. In this way, the response is tailored to DDS through PARP1.

ATM and PARP1 represent just two components of the DDR in regulation of autophagy. Several other proteins involved in the DDR regulate autophagy either directly or indirectly. Examples include members of the sirtuin family of proteins (SIRT1–7) as well as FIP200 and SQSTM1/p62. While their influence on induction of autophagy is well documented, many of the roles carried out by these proteins regarding autophagy fall out of the realm of the DDR [110–113]. For this reason, interested readers are referred to the recent review by Czarny and Blasiak for more information [113].

6.3.2 Inflammation

Another cellular response elicited by the DDR is the induction of inflammatory cytokines through activation of NF-κB. Following various types of DNA damage, ATM and PARP1 play a synergistic role in the activation of NF-κB [114]. PARP1 functions to upregulate gene expression of NF-κB as well as in its direct activation. Upon DNA damage, PARP1 auto-PARylation serves as a signaling event for the recruitment of IKKγ as well as ATM and the E3-type small ubiquitin-like modifier (SUMO) ligase, PIASγ. This interaction leads to the SUMOylation of IKKγ by PIASγ at Lys277 and Lys309, resulting in IKKγ activation. Activated IKKγ stimulates NF-κB transcriptional activity and induction of proinflammatory cytokines promoting cellular survival and chemotherapeutic resistance [115]. Additionally, there is evidence that NF-κB is directly PARylated by PARP1; however, studies conflict on the exact consequence of this modification [115–117]. In addition to PARP1, ATM also plays a role in IKKγ activation through phosphorylation of IKKγ at Ser85. In conjunction with the SUMO modifications elicited by PIASγ, this promotes binding and activation of NF-κB [114]. NF-κB activity leads to the induction of BRCA2 and ATM transcription as well as the promotion of HR through enhanced DNA end resection following DSBs [118].

While the activation of NF-κB is protective, there are times were pathogenic induction of inflammation can occur following DNA damage. When exposed to chronic DNA damage, p53 is continuously activated by ATM, ATR, and DNA-PK. This can lead to the release of the proinflammatory protein HMGB1. HMGB1 has a variety of functions; however, in this context it is released as an extracellular damage-associated molecular pattern (DAMP). DAMPs activate macrophages and dendritic cells leading to the induction of TNFα, IL-1, and IL-6 [119]. This response causes a prolonged inflammatory state that can lead to both tissue injury as well as tumorigenesis [120, 121].

6.3.3 Cell Death

In cases of severe DNA damage, both cycling and postmitotic cells must have programs in place to ensure that unrepairable damaged cells do not persist to become cancerous. The most common way for cells to eliminate themselves when this occurs is to trigger apoptosis, a highly regulated and energy-dependent form of cell death. While there are many elicitors of apoptosis, this section will focus mainly on the apoptotic pathways promoted through p53 as it is the main effector protein in this process (Fig. 6.5a).

Following DNA damage and activation of apical kinases, ATM, ATR, and DNA-PK rapidly phosphorylate p53 at Ser15. While many other phosphorylation events occur upon p53, most serve to stabilize the protein and prevent it from rebinding to its negative regulator, MDM2. Ser15 phosphorylation serves to activate the

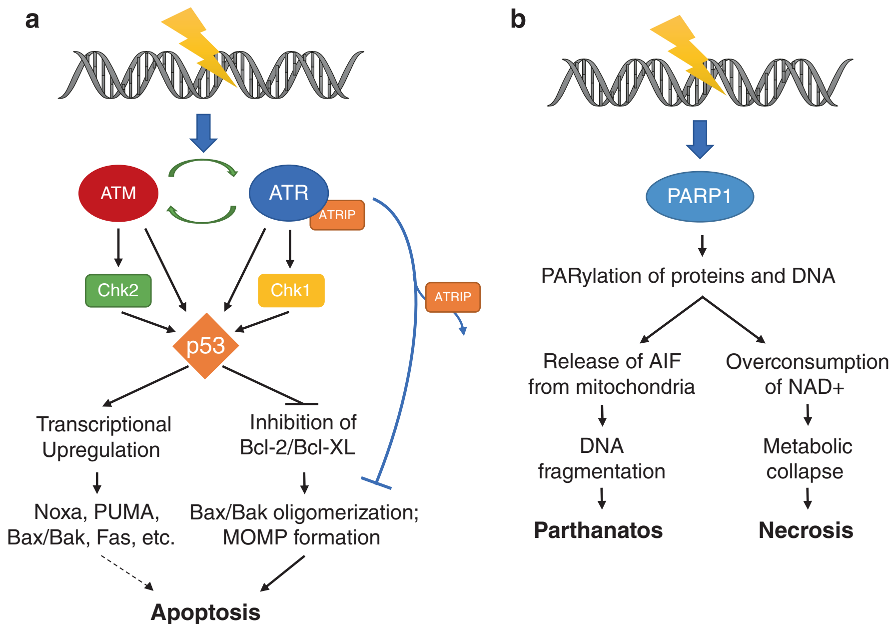

Fig. 6.5 DNA damage and cell death. Severe DNA damage can lead to the death of cells. This typically occurs through one of three mechanisms: (**a**) p53-mediated apoptosis or (**b**) PARP1-mediated parthanatos or necrosis. p53 can mediate apoptosis either directly or through induction of gene transcription where it upregulates proteins involved in both the intrinsic and extrinsic apoptosis pathways. In contrast to the regulated forms of death carried out by p53, PARP1 mediates two versions of cell death that have little order. The first is parthanatos which involves export of AIF from the mitochondria where it is then imported into the nucleus and causes non-specific DNA fragmentation. The other, necrosis, is highly unregulated and is based on the overconsumption of NAD^+ leading to decreased ATP production and metabolic collapse

transcriptional activity of p53, leading to upregulation of several pro-apoptotic genes: Bax/Bak, Puma, Noxa, Fas, etc. [122, 123]. Puma and Noxa, both members of the Bcl-2 family, serve to disrupt the binding of Bax and Bak to their respective negative regulators Bcl-2 and Bcl-XL. This allows for Bax and Bak to induce mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c through self- and hetero-oligomerization in MOMP complex formation. These events lead to rapid apoptosome formation, culminating in apoptosis [124, 125]. Fas-ligand receptor, a pro-apoptotic receptor, also is upregulated upon p53 transcriptional activation. This and other upregulated pro-death receptors serve to facilitate p53-dependent extrinsic apoptosis following DNA damage [125]. This is in addition to the transcriptional activities of p53 listed previously in Sect. 2.1.

As well as transcriptional upregulation, p53 associates through its DNA-binding domain with mitochondria where it directly promotes MOMP. This occurs through p53 displacement of Bcl-2 and Bcl-XL from Bax and Bak, respectively, and is independent of p53's transcriptional activity. It is thought, in this way, to act as a pro-apoptotic BH3-like protein in its disruption of the anti-apoptotic functions of Bcl-2

and Bcl-XL [126, 127]. As p53 phosphorylation allows p53 to change conformation and bind DNA, it is possible that ATM, ATR, and DNA-PK DDS promote this direct apoptotic function. This, however, is not without regulation as both ATR and ATM also have both direct and indirect anti-apoptotic activities [128–130].

In addition to apoptosis, cell death following extreme DNA damage can occur via autosis, parthanatos, or necrosis. Autosis, or excessive autophagy, can lead to cell death through overstimulation of the autophagic mechanisms presented in Sect. 3.1 which leads to over catabolism of cellular components and metabolic collapse [131]. Parthanatos and necrosis are two cell death processes primarily dependent on PARP1 [1, 31]. In parthanatos, PARP1 induction of PARylation serves as a signal leading to the release of AIF (apoptosis-inducing factor) from the mitochondria and its import into the nucleus. Once in the nucleus, AIF cleaves DNA in a non-specific fashion leading to its degradation and resulting in cell death. Necrosis results from PARP1 overconsumption of NAD^+ leading to a deficiency of NAD^+, loss of glycolytic capability, and, ultimately, to metabolic collapse (Fig. 6.4b) [116].

6.4 Implication in Cancer: Chemotherapeutics and the DNA Damage Response

Given the importance of the DDR in processing DNA damage, it is imperative to consider the role of DDR in response to chemotherapy. Current chemotherapeutic mechanisms range from protein inhibition to DNA-damaging agents. These can target neoplasms dependent on genetic profile and origin. Due to the unique nature of each cancer, a variety of screening techniques have been developed to identify commonly occurring mutations allowing for more specific and targeted approaches to be applied [132, 133].

The DDR is invoked following various chemotherapeutic treatments. The simplest of which are those that lead to DNA damage (Table 6.2). These are the mainstay of most treatment regimens and have long been used to treat neoplastic malignancies. Agents in this category range from base-modifying agents (alkylators and cross-linkers) to direct and indirect strand break inducers (antimetabolites, topoisomerase inhibitors, mitotic inhibitors, and radiomimetics). These trigger the DDR processes detailed previously within this chapter [133, 134]. These DNA-damaging agents, pathways activated, and repair types initiated are summarized in Table 6.2 for ease of reference.

While significantly effective treatments, DNA damage therapies are typically highly toxic. In most instances, this toxicity is not limited to just cancer cells, but also affects non-cancerous cells as well. Moreover, it is common for cancers to develop a resistance to direct DNA-damaging agents alone over the course of treatment due to acquired mutations [132, 133]. Because of this, there is a constant need for development of alternative strategies for treatment. One way forward is through profiling of mutations leading to deficiencies in various DDR pathways.

Many DDR proteins commonly contain mutations contributing to carcinogenesis. While this contribution can be through loss- or gain-of-function, the outcome is

Table 6.2 DNA-damaging chemotherapeutics and DDR

Chemotherapeutic class (representative drug)	DNA damage induced	DNA damage response pathway activated	DNA repair type
Alkylators – Alkyl sulfonates (busulfan) – Ethylenimine (altretamine, thiotepa) – Nitrogen mustards (cyclophosphamide, ifosfamide) – Nitrosoureas (lomustine, streptozocin) – Triazines (dacarbazine, temozolomide)	DNA alkylation	ATM/ATR	BER, MMR, NER
Antimetabolites/nucleoside analogues – 5-Fluorouracil – Cytarabine – Gemcitabine	Mismatch, replication stress (SSB, DSB)	ATR/ATM	BER, HR, MMR
Cross-linkers – Antitumor antibiotics (mitomycin-C) – Platinums (cisplatin, carboplatin)	Inter- and intrastrand cross-links	ATR/ATM	NER, ICLR
Topoisomerase inhibitors – Topoisomerase I (topotecan, irinotecan [CPT-11]) – Topoisomerase II (etoposide [VP-16], teniposide) – Topoisomerase II (anthracycline antibiotic, doxorubicin) – Topoisomerase II (anthraquinone antibiotic, mitoxantrone)	SSB, DSB, protein-DNA topo-adducts	ATR/ATM	NER, HR, NHEJ
Mitotic inhibitors – Taxanes (pacitaxel, docetaxel) – Epothilones (ixabepilone) – Vinca alkaloids (vinblastine, vincristine)	Mitotic collapse leading to DSB formation	ATM/ATR/DNA-PK	HR, NHEJ
Radiomimetics – Antitumor antibiotics (bleomycin, C-1027)	SSB, DSB, base oxidation	ATM/ATR/DNA-PK	BER, HR, NHEJ

always an alteration of underlying pathways resulting in genomic instability. Although these mutations, and subsequent genomic instability, contributed to carcinogenesis, they also can be exploited therapeutically. Synthetic lethality approaches aim to increase toxicity of chemotherapeutics to neoplastic cells while simultaneously reducing toxicity in non-cancerous cells by exploiting these genetic deficiencies. In many cases, some individual therapeutics without adjuvant DNA-damaging treatments can be sufficient to induce cancer cell death due to underlying genetic deficiencies [132, 134].

A good example of the synthetic lethality approach is the use of PARP1 inhibitors in BRAC1- or BRAC2-deficient cancers. Inhibition of PARP1 leads to a deficiency in BER and subsequent accumulation of SSBs either with the PARP1 inhibitor alone or in combination with additional DNA-damaging agents. This leads to persistence of SSBs, resulting in DSBs and replication fork collapse during S phase. As mentioned previously, BRAC1 and BRAC2 are necessary for HR, and, as such, without functioning BRAC1/2, HR will not occur. Thus, tumors possessing mutations in these proteins are deficient in HR and must rely on other forms of DNA repair to maintain genomic integrity when DSBs and replication collapse occur. PARP1 inhibition also leads to reduced NHEJ and an absence of alt-NHEJ, resulting in a mass accumulation of DNA damage as cells lack the ability to repair the damage. Sustained damage in this fashion leads not only to failed DNA repair, but in many instances to cancer cell death [132–134]. To explore this concept, Li et al. recently reported a way to induce "BRCAness" together with PARP inhibition to produce synthetic lethality to non-BRCA-deficient drug-resistant prostate cancers [135].

Additional synthetic lethality approaches involving the DDR are currently under investigation. One such example is ATR inhibition in combination with either ionizing radiation or cross-linking agents. Inhibition of ATR leads to a lack of cell cycle checkpoint activation as well as direct failure of HR/ICLR following DNA damage. Furthermore,

Table 6.3 Role of current and potential chemotherapeutics targeting DDR

Role of current and potential chemotherapeutics targeting DDR		
Chemotherapeutic class (representative drug)	Cellular pathway affected	Effect on DNA damage response and signaling
Checkpoint kinase inhibitors		
– Chk1 (LY2603618, MK-8776) – Chk2 (PV1019, VRX046617)[a]	ATR ATM	Cell cycle progression (mutation accumulation, mitotic catastrophe, and potentiation of DNA damage), reduced activation of Rad51 leading to HR deficiency Cell cycle progression (mutation accumulation, replication stress, and potentiation of DNA damage), reduced activation of BRAC1/2 leading to inefficient HR
Cyclin-dependent kinase inhibitors		
– CDK1 – CDK2 – CDK4/6 – pan-CDK	CCA at G2/M CCA at G1/S CCA in G1 Complete CCA	Potentiates cell cycle arrest induced by concurrent chemotherapeutic treatments, allows for the accumulation of DNA damage and pro-apoptotic signaling
DNA ligase IV inhibitor[a]		
– L189, SCR7	NHEJ	Inhibition of DNA ligase IV preventing ligation following NHEJ, prolongation of DSBs and increased ATM signaling
HDAC inhibitors[b]		

Table 6.3 (continued)

Role of current and potential chemotherapeutics targeting DDR		
Chemotherapeutic class (representative drug)	Cellular pathway affected	Effect on DNA damage response and signaling
– Class I (HDAC 1,2,3,8) – Class IIB (HDAC 6) – Class III (SIRT1, 6, 7)	NER, NHEJ, p53 MMR BER, HR, NER, Alt-NHEJ, p53	HR and NHEJ, accumulation of H3K56Ac, H4K16A, H4K91Ac preventing protein recruitment; p53, simulation of p53 transcription MMR, increases MSH2 stability leading to better detection of mismatched bases BER, controls substrate specificity of TDG and lessens APE1 activity (Sirt1), lessened activation of WRN(Sirt1), lessened activation of PARP1 (Sirt6); HR, activation of CtIP; HR/Alt-NHEJ, lessened activation of PARP1 (Sirt6); NER, lessened XPA binding to other NER factors (Sirt1); p53, potentiated p53-induced apoptosis (Sirt1)
Ku70/Ku80 inhibitors[a]		
– Vitas-M STL127705, ZINC 09009828	NHEJ	Prevention of Ku70/80 binding to DNA leading to loss of NHEJ function
MRN inhibitor[a]		
– Mirin – PFM01, PFM03, PFM39	HR, NHEJ (minor) HR	Failure of MRN activation of ATM (loss of ATM dependent signaling), inhibition of MRE11 nuclease activity Inhibition of MRE11 nuclease activity, promotion of NHEJ
RAD51 inhibitor[a]		
– B02, DIDS, RI-1, RI-2 – IBR2, IBR120	HR HR	Inhibition of RAD51 ssDNA-binding activity Inhibition of RAD51 binding to BRAC2, decrease in BRAC2 recruitment to sites of DNA damage
RPA[a]		
– TDRL551	HR, ICLR, NER	Disruption of ssDNA-binding capacity and replication, replication fork collapse, lessened recruitment of ATR-ATRIP
PIKK family inhibitors		
– ATM (KU-55933, KU-60019)[a] – ATR (AZD3738, VE-821, VE-822/ VX-970) – DNA-PK (NU7026, KU-0060648)	HR, ATM HR, ICLR, ATR NHEJ	Increased accumulation of DNA damage due to loss of cell cycle control, inhibition of HR, reduced autophagic signaling following DNA damage Increased accumulation of DNA damage due to loss of cell cycle control, loss of HR and ICLR leading to increased strand breakage and NHEJ Inhibition of NHEJ forcing the use of HR
PARP1 inhibitors		
– Oliparib, Rucaparib, Veliparib	Alt-NHEJ, BER, HR (minor), NHEJ	Alt-NHEJ and BER, failure to recruit XRCC1; HR, lessened recruitment of MRE11 and RAD51 to facilitate stalled replication fork restart; NHEJ, lessened DNA-PK activation and failure to recruit DNA ligase IV

[a]No inhibitors of this type have reached the clinical trial stage
[b]HDAC class IIA shows little effect on the DDR

inhibition of ATR forces the use of the NHEJ pathway which can lead to further accumulation of deleterious mutations as it is not a high-fidelity form of repair [136].

Many other inhibitors targeting the DDR are under development or already have reached clinical trial (Table 6.3) [132–134, 136–140]. As can be noted from Table 6.3, almost every aspect of the DDR is currently under investigation. With advanced technologies increasing tumor profiling capability, there likely will be a rise in synthetic lethality approaches using DDR proteins as targets. This will hopefully lead to increased chemotherapeutic efficiency as well as reduce off-target toxicity; both of which are essential for good patient outcome.

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