Chapter 14

Cell-Cycle Control and DNA-Damage Signaling in Mammals

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1. INTRODUCTION

For the survival and normal functionality of a mammalian organism, it is essential to ensure the maintenance of genomic integrity and the accurate transmission of genetic information between daughter cells at the cellular level. To safeguard the integrity and functionality of their genomes, mammalian cells utilize molecular mechanisms to separate the replication of DNA in S phase from the equal distribution of genetic information during M phase. Very accurate cell cycle–control mechanisms ensure that a cell does not reduplicate chromosomes before sister chromatid separation. Conversely, cells do not enter into mitosis before DNA duplication is completed. These mechanisms are orchestrated by the oscillating activities of cyclin-dependent kinases (CDKs), the master regulators of cell-cycle progression [1,2]. CDKs are regulated by complex formation with different cell-cycle phase-specific cyclins, supporting kinase activity and substrate recruitment. The activities of CDK–cyclin complexes are regulated by controlling the synthesis and degradation of cyclins throughout the cell cycle. CDK activities are further modulated by specific activating or inhibiting phosphorylations and interactions with specific CDK inhibitors (CKIs). Collectively, these events enable mammalian cells to orderly progress through the cell cycle, and hence to preserve genomic stability.

Besides faulty genome replication and/or segregation, the integrity of mammalian genomes is also threatened by spontaneous endogenous DNA damage or exogenously induced DNA lesions. Therefore, cells utilize a fine-tuned DNA-damage response (DDR) to coordinate the detection, signaling, and repair of DNA damage. The cellular DDR was initially discovered in yeast, where it functions to arrest the cell cycle in order to allow DNA repair [3]. The mammalian DDR also couples DNA repair with cell-cycle progression and DNA replication, and possibly, when the DNA damage is beyond repair, in the commitment to undergo apoptosis or terminal differentiation through senescence [4–7]. Generally, the DDR is a kinase-based signaling network. DDR signaling plays a role in the coordination of the response to different types of DNA lesions, where the role of the DDR in DNA double-strand break (DSB) repair is best understood. In response to DSBs, as one of the earliest events in DDR signaling, the DNA-damage sensor and signal mediator complex MRE11/RAD50/ NBS1 (MRN) is recruited to the sites of DNA lesions. The recruitment of the MRN complex assists the activation of ataxia telangiectasia–mutated kinase (ATM) by sequestering ATM at DSBs, which results in ATM-mediated formation of γ H2AX in the vicinity of DNA breaks up to distances of megabases. Together MRN and γ H2AX function as signal amplifiers that further enhance local ATM activation and recruit additional DDR mediators, effectors, and DNA-repair factors to DNA. In response to DNA damage, many molecular events take place involving posttranslational modifications, such as phosphorylation, ubiquitinylation, and others, which can help coordinate selective and regulatory protein–protein interactions of central DDR molecules [4–11].

By maintaining genomic integrity, the DDR is critical to prevent aging and defend against malignant transformation [5,12]. Therefore, it is not surprising that many commonly lost tumor-suppressor genes, including p53, p16^{INK4A}, RB, BRCA1/2, and ATM, play roles in the DDR which is among the most frequently compromised pathways in human cancers [13–20]. Defective DNA repair can also result in striking disease phenotypes. For example, deficiency in nonhomologous end joining (NHEJ) results in severe combined immunodeficiency (SCID) due to impaired V(D)J immunoglobulin arrangements [5]. Patients suffering from NHEJ defects are further predisposed to develop lymphoid tumors stemming from T and/ or B cells with abnormal TCR and/or V(D)J recombination [5]. As another example, mutations in ATM or components of the MRN complex have been linked to a broad range of clinical features including progressive ataxis, telangiectasia, mild immunodeficiency, and cancer predisposition [19,21–26]. Generally, DDR defects can result in cancer predisposition, neurological disorders, premature aging, impaired immune biology, infertility, and other syndromes [5]. Specifically, since genome instability is a fundamental feature of cancer [27–29], and DNA-damaging agents, such as cisplatin, doxorubicin (Adriamycin), and ionizing radiation (IR) are routinely used in chemotherapy regimens [30,31], the bettering of our understanding of DNA-damage signaling has gained an extensive interest in order to improve DNA-damaging cancer therapies with the aim of reducing the frequency of cancer therapy resistance [32].

Here, we provide an overview of key molecular events in the control of the mammalian cell cycle with a particular emphasis on cell cycle–checkpoint activation and DNA-damage repair in response to DNA damage.

2. CELL-CYCLE PROGRESSION IN MAMMALIAN CELLS

To progress through the cell cycle in a coordinated fashion is a complex challenge for every eukaryotic cell, particularly in multicellular organisms with many specialized cell types. To ensure genomic integrity, a cell must be able to detect DNA lesions followed by signal integration to establish checkpoints which are specific for each cell-cycle stage, allowing sufficient time for various types of DNA repair.

2.1 Definition of Cell-Cycle Phases

The faithful transmission of genetic information between daughter cells is performed by two central processes: DNA replication and cell division. The mammalian cell cycle can be subdivided into four different stages occurring in the following order: G1, S, G2, and M phases (Fig. 14.1). G1, S, and G2 are referred to as interphase, while the M phase is also known as mitosis. During the first gap phase (G1), the cell prepares for a new round of duplication, ensuring that sufficient building blocks and the right environment/conditions are available for a successful cell multiplication. In case conditions are unfavorable, the cell halts in a transient G1 arrest, activates reversible cell cycle–exit mechanisms to enter G0 (quiescence), or commits to a permanent cell-cycle arrest (senescence). During the S phase, DNA synthesis takes place to completely and accurately replicate the double-stranded DNA molecules. During the second gap phase (G2), the cell prepares for the mitotic division. In mitosis, divided into prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis, the cell divides by segregating the chromosomes into two separate daughter cells. Collectively, during cell-cycle progression, a mammalian cell ensures to precisely replicate (copy) its genetic information in the S phase and to proper partition (distribute) chromosomes between daughter cells in the M phase. Equally important, the cell can identify and correct DNA lesions that can arise spontaneously or are induced exogenously. In this regard, the cell can activate cell-cycle checkpoints to arrest cells in the G1, S, and G2 cell-cycle phases (Fig. 14.1).

2.2 Molecular Regulation of Cell-Cycle Progression

The coordinated progression through all four phases of the cell cycle is dependent on protein phosphorylation [33,34]. The serine/threonine CDKs form catalytically active heterodimer complexes with cyclins which can be regulated by CKIs, such as p21 (CIP1/WAF1) or p27 (KIP1) [35]. The oscillating activities of CDKs are essential to safeguard the timely duplication and segregation of the genome (Fig. 14.1). Different CDKs contribute to cell-cycle progression: CDK2, CDK4, and CDK6 are active in G1, CDK2—during the S phase, and CDK1—during the G2 and M phases. The transition from G1



FIGURE 14.1 Regulation of the mammalian cell cycle by CDK-cyclin complexes. Different cyclin-dependent kinases (CDKs) play roles in regulating cell-cycle progression. In G1, cells can reversibly exit the cell cycle and enter into quiescence (G0) or permanently exit the cell cycle by entering into sensecence. Alternatively, a mammalian cell can decide to commit to another cell cycle round. In this case, CDK4/6-cyclin D complexes in early G1 and CDK2-cyclin E complexes in late G1 phosphorylate pRB to promote the release of E2F which enables progression into the S phase. The progression through the S phase is promoted by phosphorylations performed by CDK2-cyclin E and CDK2-cyclin A complexes. In G2, the CDK1-cyclin A complex initially promotes cell-cycle progression, while in late G2, the CDK1-cyclin B complex takes over. The entry into mitosis (M) is triggered by a sharp increase in CDK1-cyclin B activity. Note that, if needed, mammalian cells can activate cell-cycle checkpoints in all four cell-cycle phases (indicated by *red lines*).

to S is initiated by phosphorylations performed by CDK4/6–cyclin D and CDK2–cyclin E complexes [33,36]. In normal conditions, the retinoblastoma protein (pRB) is phosphorylated by CDK4/6-cyclin D in early G1 and CDK2-cyclin E in late G1. Fully phosphorylated pRB then releases the E2F transcription factor which enables S-phase progression [36–42]. Progression through the S phase is subsequently coordinated by phosphorylations performed by CDK2–cyclin E and CDK2–cyclin A complexes. Protein phosphorylation is also crucial for the G2/M transition and in M-phase progression regulated by CDK1–cyclin A and CDK1–cyclin B complexes together with other mitotic kinases, such as Aurora, PLK, NEK, and Greatwall [43]. During the normal cell-cycle progression, the entry into mitosis is triggered by a sharp increase in CDK1–cyclin B activity, which requires the removal of inhibitory phosphorylations on CDK1 generated by Wee1 and Myt1 [44]. Members of the CDC25 family of phosphatases are responsible for the removal of these inhibitory phosphorylations. Activating phosphorylations are added to CDC25 by PLK1, MAPKs, and CDK1, while inhibitory phosphorylations are added to CDC25 by CHK1, CHK2, and MK2 (MAPK-activated protein kinase 2) in response to DNA damage [8]. In particular, the CDC25A phosphatase needs to be tightly regulated in the context of the G1/S and intra-S-phase checkpoints (see later).

To prevent the inappropriate cell-cycle progression, the activity of CDKs and other cell-cycle kinases has to be tightly regulated at the G1/S and G2/M transitions. This is mainly achieved by specific interactions with CKIs and by a direct phosphorylation and/or dephosphorylation of kinases and regulatory cyclin subunits. CDKs are activated by T-loop phosphorylation mediated by CDK7-cyclin H. CDKs can be inhibited by Myt1- and Wee1-mediated phosphorylation which can be removed by CDC25 phosphatases [45–47]. Furthermore, two main protein families function as CKIs: (1) the INK4 family (composed of p15, p16, 18, and p19) inhibits CDK4/6 activity by preventing CDK4/6–cyclin D complex formation, and (2) the WAF proteins p21, p27, and p57 inhibit primarily CDK2–cyclin E and CDK4–cyclin D complexes. Another level of CDK regulation is subcellular localization. For example, the regulated translocation of CDK1–cyclin B complexes from the cytoplasm to the nucleus promotes mitosis [47,48]. E3 ubiquitin ligases also play key roles in cell-cycle progression. E3

ligases can mark their substrates by promoting the addition of polyubiquitin chains which targets the substrate for destruction by the proteasome. For example, CKIs p21 and p27 are regulated by phosphorylation-mediated degradation through the SCF^{SKP2} E3 complex, a major cell cycle–E3 ligase [49,50]. The APC/C, the second major cell cycle–E3 ligase, marks SKP2, cyclin A, and cyclin B for degradation to regulate CDK1 and CDK2 activities in the G1, G2, and M phases [51]. Specifically, the degradation of SKP2 counteracts the unscheduled degradation of SCF^{SKP2} substrates in G2, while the timely degradation of cyclin A and cyclin B is crucial for normal progression through G2/M and M. Upon G2/M transition, CDK2 activity is blocked by SCF^{FBW7}-mediated cyclin E degradation, and CDK1 activation is assisted by SCF^{βTrCP}-mediated degradation of Wee1.

Intriguingly, several of these regulatory events are linked to DNA-damage signaling by proteins that preferentially bind to phosphorylated serine and/or threonine residues. These phospho-binding proteins function together with cell cycle-checkpoint kinases to control cell-cycle progression [8]. In this regard, it is noteworthy that upon recovery from DNA-damage checkpoints, the polo-like kinase 1 (PLK1), the Wip1 phosphatase and other kinases and phosphatases play key roles [52,53]. Wip1 can suppress the phosphorylation of key DDR factors, such as p53 and ATM [52,53], while PLK1 is required for CDC25 activation to initiate mitosis as well as the progression through and exit from mitosis. In response to DNA damage, CHK2 inhibits PLK1 by phosphorylation. Conversely, upon completion of DNA repair, PLK1 shuts off ATM/ATR-CHK1/2 signaling to silence the G2/M checkpoint to allow mitotic entry. Thus, PLK1 plays a role as a down-stream target and an upstream regulator of the DDR pathway.

In summary, in the basal state, CDK–cyclin complexes are ready to promote cell-cycle progression upon removal of their inhibitory modifications and/or interactions. CDK activation and consequently cell-cycle progression occur when the inhibitory phosphates are removed by CDC25 phosphatases, and inhibitory kinases and CKIs are inactivated, destroyed, or sequestered away from CDK–cyclin complexes.

3. DNA-DAMAGE SIGNALING AND REPAIR IN MAMMALS

Mammalian genomes are under constant assault from endogenous and exogenous DNA lesions [54,55] where endogenous DNA damage can be caused by defective/stalled DNA replication, reactive oxygen species, and other mechanisms [56]. DNA DSBs are believed to be the most harmful forms of DNA damage [57]. DSBs can occur accidentally during normal DNA metabolism [56] or after exposure to exogenous agents, such as IR or DNA-damaging chemotherapeutics during radio- or chemotherapy [31,58]. To counteract DNA damage, cells make use of an array of signaling and repair mechanisms, involving kinases, nucleases, topoisomerases, helicases, ligases, polymerases, glycosylases, and other enzymes. Once DNA lesions have been detected, DDR pathways can promote different outcomes depending on the severity of DNA damage and the cell type. Possible outcomes include slowing down or arresting cell-cycle progression, permanent cell-cycle arrest (senescence), or the initiation of apoptotic programs. In either case, the aim is to prevent the replication of damaged DNA and the inheritance of DNA damage by daughter cells.

Various factors involved in cell-cycle control, DDR signaling, and DNA-damage repair contain specific modular domains, including the phospho-binding BRCT and FHA modules that play important roles in regulatory protein–protein interactions in the DDR [8]. In this regard, the actions of protein kinases are linked to physiological events by controlling specific phosphorylation-dependent protein–protein interactions. In mammalian cells, the ATM-CHK2 and ATR-CHK1 kinase cascades are the two major types of DDR kinase signaling. The ATM and ATR (ATM and Rad3-related) kinases are members of the phosphatidylinositol 3-kinase-related kinases (PIKKs) family of serine/threonine protein kinases [59]. The DNA-dependent protein kinase (DNA-PK) is another member of the PIKK family [60] regulating DSB repair together with ATM (see later). Noteworthy here is, although ATM is the central DDR kinase, most ATM substrates are still phosphory-lated to a certain degree in ATM-deficient cells in response to DNA damage, suggesting that other PIKKs, such as ATR and DNA-PK, are also important for DDR signaling [61].

3.1 DDR Signaling

Generally, DNA-damage signaling can be subdivided into four levels (Fig. 14.2): (1) DNA-damage detection by sensors, such as the MRN and Ku70/Ku80 complexes, (2) the formation/recruitment of mediators, such as γ H2AX and MDC1, (3) the activation of signal transducers, such as the ATM and ATR kinases, (4) the activation of effectors, such as p53 and others (see later). In response to DNA damage, different factors, such as the MRN complex, PARP1, and Ku70/Ku80 bind rapidly to sites of DNA damage resulting in DDR activation [4–7,62]. In particular, DSBs trigger a range of signal transduction processes with the ATM-CHK2 pathway as a primary response, while the ATR-CHK1 pathway tends to be activated by exposure to ssDNA, stalled replication forks, and bulky DNA base adducts [4,5,7,61,63]. However, ATR can



FIGURE 14.2 Main steps of DNA damage–response signaling. DNA-damage signaling can be subdivided into four levels: the detection of DNA damage (eg, DSBs) by DNA-damage sensors (eg, MRN and Ku70/Ku80 complexes), the formation/recruitment of signal mediators (eg, γ H2AX and MDC1), the activation of signal transducers (eg, ATM and ATR kinases which are mainly activated by DNA breaks), and the activation of effectors (eg, p53 and others). Note that signal mediators can facilitate the amplification of the initial DNA-damage signal. Signal transducers can also act in positive-feedback loops by promoting the activation/recruitment of DNA-damage sensors and signal mediators, resulting in a further amplification of the initial DNA-damage signal. For a more complete overview of these DNA damage–signaling steps, please refer to the main text.

also be involved in DSB signaling [4–7]. Specifically, ATM-mediated DSB resection and the consequent single-stranded DNA (ssDNA) formation also promote ATR/CHK1 signaling in S and G2 [64,65]. Thus, the ATM response to DSBs is very rapid and cell-cycle independent, while the ATR response is generally slower and requires CDK-dependent resection, hence being restricted to S and G2. The general view is that the checkpoint kinases CHK1 and CHK2 are key effectors of ATR and ATM signaling. CHK2 is activated by ATM in a cell cycle–independent manner, while CHK1 is activated by ATR following ATM and MRN-mediated resection of DSBs in a cell cycle–dependent fashion. As a third main regulator of DNA damage–checkpoint activation, the p38MAPK/MK2 pathway can function downstream of ATM and ATR in response to DNA damage [66,67]. Here, we provide an overview of the key sensors, mediators, transducers, and effectors in mammalian DNA-damage signaling (Fig. 14.2).

The MRN complex functions as the initial DSB sensor tethering the broken DNA ends together [24–26]. The interaction between NBS1 and ATM [8] results in the initial sequestering of ATM to DSB sites [24–26]. Once activated by autophosphorylation [68], ATM phosphorylates many different substrates involved in DDR signaling [19,61]. On the one hand, the activated ATM phosphorylates the MRN component NBS1 to create a positive-feedback loop maintaining/amplifying ATM activity [61]. On the other hand, the ATM-mediated phosphorylation of the H2AX histone results in the formation of γH2AX which consequently accumulates in the vicinity of DSBs. The formation of γH2AX foci initiates the recruitment of other DDR factors to sites of DNA lesions. γH2AX formation is normally ATM dependent but can also be mediated by DNA-PK or ATR [61]. Note that in addition to phosphorylation, other posttranslational modifications, such as ubiquitylation and methylation are also involved in the early steps of DDR signaling [4–11]. In a nutshell, γH2AX formation recruits MDC1. MDC1 in turn interacts with MRN and ATM to tether MRN and ATM at DSBs, and also recruits the RNF8 (RING finger 8) and RNF168 E3 ligases which ubiquitylate H2A in the DSB vicinity. H2A ubiquitylation then influences the methylation status of other histones which can promote 53BP1 recruitment to DSBs, thereby influencing the choice of DSB repair (see later). Note that additional factors can be sequestered at DSBs in a cell cycle–dependent manner, including BRCA1 and other protein complexes [69]. However, since the phosphorylations mediated by the activated ATM are crucial for DDR signaling, we focus here on discussing key substrates of ATM in the DDR [61].

ATM-mediated γ H2AX formation serves as a platform for the recruitment of DDR factors and as an amplifier of the initial signal. However, γ H2AX deficiency has only subtle effects on cell-cycle checkpoints and DNA repair, indicating that γ H2AX may only regulate repair and signaling of a portion of DSBs [61]. In addition, in mammalian cells, γ H2AX-foci preferentially form in euchromatin, being mostly excluded from densely packed heterochromatin [70], suggesting that DNA lesions in heterochromatin are signaled by γ H2AX-independent mechanisms.

The transcription factor p53 is a major effector of ATM signaling [36,61,71] mediating a G1 cell–cycle arrest mainly through the transcriptional upregulation of p21 [72]. Alternatively, if the DNA damage is too extensive, p53 triggers cell death through intrinsic and extrinsic pathways [73,74]. Generally, p53 as a downstream effector of ATM signaling acts as a major DNA damage–checkpoint regulator (see Chapter 15).

ATM also phosphorylates the checkpoint regulator CHK2. Once activated by ATM, CHK2 acts as an effector kinase by phosphorylating numerous downstream targets, including CDC25, p53, and BRCA1 [75]. Of note, ATM-mediated phosphorylation of CHK2 is followed by additional autophosphorylation events which are required for the full activation of CHK2 (summarized in [8]). In general, CHK2 is a key effector downstream of ATM signaling in the DDR.

ATM also phosphorylates the KAP1 (Krüppel-associated box (KRAB)-associated protein 1) [76] which allows the transient and localized relaxation of heterochromatin without affecting epigenetic marks in the vicinity of DSB [77–80]. Specifically, ATM-dependent KAP1 phosphorylation is essential for DSB repair within heterochromatin regions. This function is important since the connection between chromatin architecture and DNA-damage signaling can modulate the choice between different DNA-repair pathways (see later).

ATM further phosphorylates the structural maintenance of chromosomes 1 protein (SMC1) known to function in complex with SMC3 in sister chromatid cohesion and DNA recombination. This phosphorylation of SMC1 by ATM can play a role in the intra-S-phase checkpoint, hence being required for genomic stability and consequently cell survival in response to DNA damage [81–83].

Note that ATM also phosphorylates factors involved in the repair of DSBs, including CtIP, BRCA1, and RAD51 [61] which can influence DSB-repair pathway choice (see later). In this regard, it is most likely that in response to DNA damage, the ATM/ATR kinases may phosphorylate more than 700 different substrates in mammalian cells [84]. This underscores the central importance of ATM in the DDR, while highlighting the challenges that lie ahead to functionally decipher the importance of ATM- and/or ATR-mediated phosphorylation events. Last, but not least, one should also note that defective ATM activation is quantitative, not absolute, in MRN mutant cells [22,61,82,85,86], and MRN-mediated ATM activation can be dispensable for p53 and CHK2 phosphorylation [23,87]. This suggests, on the one hand, that ATM can be activated through different signaling routes, while, on the other hand, not all known ATM substrates may represent suitable readouts for ATM activity in certain settings.

3.2 DNA-Damage Repair

Tens of thousands of DNA-damaging events take place in every cell on a daily basis [54–56]. Thus, DNA lesions are recognized and processed by highly specialized DNA-repair systems to ensure a quick and accurate removal of DNA damage [4–7]. These systems are crucial since the persistence of DNA mutations can result in the altered gene functions potentially causing cancer development, tissue degeneration, and other human diseases [5,88]. Specifically, the defective DNA repair can lead to elevated mutation rates, which when occurring in tumor-suppressor genes or proto-oncogenes can cause cancer development, as, for example, is the case in mismatch repair (MMR) defects [89–92]. Defects in HRR can also predispose patients to cancer considering the data of heterozygous carriers with mutations in BRCA1/2, two HRR components that function as tumor-suppressor proteins [14]. In addition, human diseases can derive from deficiencies in DSB-repair pathways, exhibiting premature aging, cancer predisposition, and defects in neurobiology, immunology, and development [5,88,93–98].

Since DSBs represent very dangerous DNA lesions that will cause an uneven division of the genome during the M phase when not dealt with prior to mitotic entry, we focus our overview of DNA damage–repair mechanisms on DSB-repair pathways in the context of cell-cycle dependencies. Nonetheless, we also summarize other DNA-repair mechanisms, such as nucleotide excision repair (NER), base excision repair (BER), and MMR which are defined in more detail elsewhere [99]. Interstrand crosslink (ICL) repair is not discussed since it is primarily performed by a combination of NER, HRR, and other repair pathways.

3.2.1 Nucleotide Excision Repair

NER mainly fixes "bulky" DNA alterations including ultraviolet light (UV)-induced photoproducts, base adducts created by genotoxic agents, such as cisplatin, reactive oxygen species (ROS)-induced base modifications, and others [99]. NER occurs in four main steps: (1) DNA-damage recognition, (2) incision on both sides of the DNA lesion and removal of the damaged DNA fragment, (3) gap-filling DNA synthesis, and (4) ligation of open DNA ends. About 30 proteins function in the NER pathway, and defects in NER components are linked to human diseases, such as xeroderma pigmentosum, Cockayne syndrome, and others [99]. Generally, the NER pathway can be subdivided into two processes: (1) the global genome NER (GG-NER) functioning in a cell cycle–independent manner to remove UV-induced photoproducts and other "bulky"

lesions, and (2) transcription-coupled NER (TC-NER) recognizing RNA polymerase stalled at "bulky" DNA lesions. GG-NER and TC-NER differ at the step of DNA recognition damage and share the remaining DNA-repair machinery [99].

3.2.2 Base Excision Repair

BER fixes nonbulky DNA base damage, abasic sites, and DNA single-strand breaks (SSBs) throughout all stages of the cell cycle [99]. BER occurs in five major steps: (1) recognition and excision of a damaged base, (2) incision at the abasic site, (3) replacement of the excised DNA nucleotide, (4) processing of DNA ends, and (5) sealing of the DNA nick. DNA gly-cosylases are responsible for the recognition and hydrolysis of DNA lesions followed by DNA polymerase β and XRCC1-ligase III-mediated nucleotide replacement and DNA nick sealing [99]. SSBs are detected by PARP1 which catalyzes the formation of poly-ADP-ribose (PAR) chains on itself and other proteins to facilitate the recruitment of specialized BER enzymes and DNA-repair factors, such as XRCC1, DNA polymerase β ,Ligases I and III [99].

3.2.3 Mismatch Repair

MMR is responsible for the recognition and repair of base–base matches and insertion–deletion loops (IDLs) which are caused by faulty DNA replication and homologous recombination [99,100]. To preserve genome integrity, MMR must take place selectively on the newly synthesized DNA strand containing the error. MSH2-containing complexes recognize DNA lesions followed by the recruitment of MLH1/3 and PMS1/2 complexes, and then the endonucleases PMS2 and MLH3 make an incision at the site of the DNA lesion. Upon the marking of the appropriate strand by incision, the exonuclease Exo1 generates a multi-nucleotide gap which is filled and ligated by DNA polymerase δ and Ligase I [99,100]. Significantly, MMR increases DNA replication fidelity by about 100-fold.

3.2.4 DSB Repair

DSBs are severe lesions that can result in the acquisition of disease-promoting properties or premature cell death when not repaired properly [5,30,31,88]. To minimize the impact of DSBs, mammalian cells utilize different DSB-repair pathways [69,99]. The two major pathways are: more error-prone but fast DNA nonhomologous end joining (NHEJ) and error-free but slow HRR. Depending on the origin of DSBs, mammalian cells use different DSB-repair mechanisms. DSBs can arise during programmed DNA recombination (eg, V(D)J and class switch recombination upon immunoglobulin production) or accidental DNA breakage upon the arrest or stalling of DNA replication and exposure to DNA-damaging agents, such as IR or topoisomerase poisons. These events cause DSBs of a distinctive nature which are recognized and processed differently [69]. The regulated resection of DSBs represents a key step in the choice between NHEJ and HRR [11,101] where the accumulation of 53BP1 at DSBs can block the resection and consequently RAD51 loading, hence influencing DSB-repair pathway choice [11,102]. Thus, 53BP1 generally promotes NHEJ and restricts HRR. Specifically, 53BP1 recruitment of RIF1 promotes NHEJ, while by excluding 53BP1 from DSB sites, BRCA1 can promote HRR [103–109].

Mostly, NHEJ occurs independently of ATM signaling, and ATM signaling takes place independently of the NHEJ machinery. DNA-PK signaling is essential for NHEJ, and ATM signaling is essential for HRR. Note that in mammalian cells, about 80% of radiation-induced DSBs are repaired quickly, while 20% of DSBs are repaired slowly [110,111]. Considering further that ATM is required for the slow repair of DSBs [111], the majority of DSBs are repaired by NHEJ. Actually, NHEJ is recognized as the predominant DSB-repair pathway in G1 and G2 [69,112]. In G1 and G2, NHEJ promotes a fast DSB repair, while in G2, but not G1, a slow process occurs by HRR. In the current DSB-repair model, NHEJ makes the first attempt to repair DSBs, but when a rapid repair does not ensue, resection occurs, thereby committing to HRR as a slow process [69,113]. In this context, one should also note that the chromatin status is very likely to influence the choice of DSB repair since euchromatin is more accessible than highly compacted heterochromatin [69]. Therefore, ATM also regulates factors such as KAP1 to promote the repair of DSBs located in heterochromatin (see earlier). Not surprisingly, other regulators of chromatin assembly influence the efficiency of DSB detection and repair as well [69,114].

Generally, major determinants of DSB-repair pathway choice are the extent of DNA end processing and cell-cycle position. The chromatin structure also contributes to this choice, with studies since 2000 suggesting that HRR is predominantly used for DSB repair in the areas of heterochromatin [111–113]. Initially, the MRN and Ku70/Ku80 complexes recognize DSBs, but they trigger different repair mechanisms (see later).

3.2.4.1 Classical Nonhomologous End-Joining Repair

NHEJ functions throughout the cell cycle as a predominant DSB-repair mechanism in mammalian cells [69,115–117]. NHEJ occurs via three main steps: (1) DSB recognition, (2) processing of nonligatable DNA termini, and (3) joining of

two suitable DSBs. Noteworthy here, NHEJ can also directly religate the broken DNA ends and does not require DNA end resection for repair initiation. Classical NHEJ (c-NHEJ) is mediated by the Ku70/Ku80 heterodimer which binds to DSBs within seconds and dictates NHEJ pathway choice [118]. This binding protects DSB from degradation and recruits DNA-PK [60] whose autophosphorylation is essential for c-NHEJ (summarized in [69]). After DSB end-processing, Ligase IV functions as an NHEJ-specific ligase supported by XRCC4 and XLF [69].

3.2.4.2 Homologous Recombination Repair

HRR is dependent on the availability of an undamaged DNA template to restore sequence information accurately; hence, HRR only functions in late S and G2 phases when a sister chromatid is available. HRR requires DNA end resection of the DNA break to initiate repair. HRR occurs via six different steps [69]: (1) generation of 3' ssDNA—also known as 5'-3' resection, (2) RPA coating of ssDNA, (3) BRCA2-assisted displacement of RPA by RAD51 to form RAD51 filaments, (4) strand invasion resulting in heteroduplex and Holliday junction formation, (5) branch migration, and (6) resolution. Current evidence suggests that resection (ssDNA generation) involves two steps, with CtIP and MRE11 functioning as initiators and other exonucleases performing the elongation [69,101]. The MRN/CtIP complex is not only important for the initiation of resection [101] but also represents the event that commits to HRR [113,119]. Noteworthy being, CDK-mediated phosphorylation of CtIP promotes DSB resection in a cell cycle–dependent manner [120], and BRCA2 phosphorylation by CDK inhibits the BRCA2/RAD51 interaction [121], suggesting that CDK activity can regulate different steps of HRR. CtIP function is also regulated by ATM phosphorylation [122], with CtIP-dependent DNA resection selectively promoting HRR while suppressing NHEJ [123].

3.2.4.3 Alternative DSB-Repair Mechanisms

Two other DSB-repair pathways are microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA). Both processes require DNA end resection for repair initiation [69,99].

MMEJ can be dependent on c-NHEJ and alternative NHEJ (alt-NHEJ). MMEJ uses short homologous sequences (microhomologies) to align the broken ends prior to ligation. Alt-NHEJ does not utilize c-NHEJ factors, such as Ku70/Ku80 and Ligase IV, but rather utilizes PARP1, XRCC1, Ligase I/III, and potentially the MRN complex [69]. Sometimes alt-NHEJ and MMEJ are used mistakenly as synonyms [69]. However, alt-NHEJ most likely represents only one variant of different MMEJ processes [69], likely as a back-up mechanism for c-NHEJ since alt-NHEJ does not seem to be active unless c-NHEJ is defective [99]. In general, alt-NHEJ is more error-prone than c-NHEJ [99]. Nonetheless, a better understanding of the alt-NHEJ-repair pathway is likely to further improve our treatment options of cancers since the inhibition of the alt-NHEJ pathway selectively sensitizes leukemia cells to cytotoxic agents [124].

SSA repair can take place when the DSB is flanked by repetitive homologous nucleotide repeats on both sides [99]. This allows the resected DSB ends to anneal with each other instead of invading a homologous DNA sequence elsewhere, as it occurs in HRR. Thus, in contrast to HRR, SSA repair does not involve strand invasion and does not require sister chromatid exchange. Consequently, SSA repair functions independently of RAD51, while being facilitated by RPA and other HHR factors [99]. Overall, in contrast to HRR, SSA repair results in the deletion of DNA sequence and is therefore error prone.

4. CHECKPOINT CONTROL: DNA-DAMAGE SIGNALING AND THE MAMMALIAN CELL CYCLE

Healthy mammalian cells respond to DNA lesions by activating DNA-damage checkpoints which delay cell-cycle progression while promoting DNA-repair mechanisms [45,125,126]. Cell cycle–checkpoints function as regulators of the cell-cycle machinery in response to DNA damage and act as a guard against the propagation of damaged DNA [127]. Considering the importance of the cellular response to DSBs, the most toxic DNA lesions [57], we focus here on defining DNA damage–checkpoint signaling in response to DSBs [128].

Three different and major cell cycle–checkpoints function in the DDR: the G1/S, intra-S phase, and G2/M DNA-damage checkpoints (Fig. 14.3). These checkpoints are primarily controlled by the signal transduction pathways that coordinate DSB recognition and repair, namely the ATM/ATR kinases. In response to DSBs, ATM and ATR initiate phosphorylation cascades that result in cell-cycle arrest at DNA-damage checkpoints where many DNA-damage checkpoints are mediated by the activation of the p53 tumor-suppressor protein [33,125,129]. Generally, DNA-damage checkpoints can be subdivided into initiation, maintenance, and recovery phases. In particular, following DNA damage, the initiation and maintenance of DNA-damage checkpoints is tightly regulated by ATM/ATR-mediated signaling (Fig. 14.3). Of course, checkpoint release and cell-cycle reentry following DNA repair must also be coordinated accordingly [52,125].



FIGURE 14.3 The main molecular players in DNA-damage cell-cycle checkpoints. In response to DNA damage, mammalian cells can activate three major DNA-damage checkpoints: G1/S, intra-S-phase, and G2/M checkpoints. DSBs trigger ATM-CHK2 kinase signaling which can block G1/S progression by promoting the p53–p21 axis and by inhibiting CDC25. The activated ATR-CHK1 kinase signaling can inhibit CDC25 as a part of the intra-S-phase checkpoint. ATM/ATR-CHK1/2 signaling can further halt cells at the G2/M checkpoint through different signaling routes as indicated. For a more complete summary of the functions of these key molecular players, please refer to the main text.

Here, we focus on summarizing the importance of ATM-CHK2 and ATR-CHK1 signaling in DNA-damage checkpoints. In this regard, one should note that the p38/MK2 pathway can complement the well-established ATM and ATR signaling nodes by converging on the regulation of CDC25 in the DNA-damage checkpoints [67,130,131]. Moreover, it is noteworthy that in response to DSBs, the efficiency of CDK1 activation as a part of DNA-damage checkpoints appears to be cell-cycle dependent since the DSB response is less efficient in G1 than in S/G2 [122].

4.1 The G1/S Cell-Cycle Checkpoint

The G1/S checkpoint is activated by very low numbers of DSBs, possibly one DSB being sufficient for the activation [132]. Two different processes primarily contribute to the activation of the G1/S checkpoint in response to DNA damage [75,126].

The first widely studied process involves the ATM-p53-p21 signaling axis [126,133]. Since this process requires the p53-dependent transcription of the CDKI p21, the entry into the S phase is only inhibited several hours after DNA damage has occurred [132]. The first G1/S checkpoint is mainly mediated by ATM, which results in the phosphorylation and activation of the p53 transcriptional activity. Consequently, the CKI p21 is upregulated in a p53-dependent

manner which causes the p21-dependent inhibition of G1 and early S-phase CDK–cyclin complexes, finally resulting in the accumulation of hypo-phosphorylated pRB which can sequester E2F and consequently block S-phase initiation/ progression [33,39].

In contrast, the second G1/S checkpoint does not fully prevent S-phase entry. Similar to the G2/M checkpoint (see later), it involves ATM/ATR activation of CHK1/2 which can result in CDK2 inhibition [132]. In this regard, the CDC25A phosphatase following phosphorylation by different kinases can be targeted for ubiquitin-mediated degradation by the SCF^{β TrCP} E3 ligase in G1 (summarized in [8]).

In general, the limitation of the G1/S checkpoint is a slow and incomplete block of entry into the S phase since this cell-cycle arrest is mainly transcription dependent [132]. Consequently, some cells can escape from G1 to S phase with unrepaired DSBs, which can cause chromosome breakage in G2 [132].

4.2 The Intra-S-Phase Cell–Cycle Checkpoint

The intra-S-phase checkpoint is important to prevent the progression of DNA replication in the presence of DSBs [134]. In particular, the execution of CDK1-dependent events can be detrimental when the intra-S-phase checkpoint is not functional since the incompletely replicated chromosomes might be missegregated in mitosis. Therefore, similar to the G1/2 checkpoint, the intra-S-phase checkpoint can be divided into two distinct processes, involving, on the one hand, the arrest of ongoing replication fork progression, and on the other hand, the inhibition of late firing replication origins. More specifically, in the S phase, radiation-induced DNA damage slows down DNA synthesis by two ATM-dependent pathways: ATM-NBS1-SMC1 and ATM-CHK2-CDC25A signaling [33,135], with the latter pathway negatively regulating DNA replication by preventing loading of the replication factor CDC45 onto replication origins [136]. ATR-CHK1 signaling also prevents new replication origins from firing during the S phase [63]. Moreover, as part of the intra-S-phase checkpoint, the phosphorylation of CDC25A by CHK1 results in the rapid SCF^{βTRCP}-mediated proteasomal degradation of CDC25A which blocks the removal of inhibitory phosphorylations on CDKs [137–139].

4.3 The G2/M Cell–Cycle Checkpoint

The G2/M checkpoint has a defined threshold of sensitivity since it is estimated that at least 10 to 15 DSBs are required for the efficient checkpoint activation and maintenance [45,69]. Therefore, the G2/M checkpoint is considered inefficient to maintain genomic stability upon deregulation of the G1/S checkpoint [45].

In healthy cells, the activation of the G2/M checkpoint in response to DNA damage prevents the entry of cells into mitosis, thereby blocking the propagation of damaged DNA to daughter cells. The activation of the G2/M checkpoint involves the activation of CHK1/2 by ATM/ATR-mediated phosphorylation. The activated CHK1/2 phosphorylates CDC25 causing CDC25 inhibition by the cytoplasmic sequestration of CDC25. Consequently, CDK1 remains inactive by sustained inhibitory phosphorylation by Wee1, resulting in the rapid inhibition of entry into mitosis [125,137,140]. At first, ATM functions as the initiator of the G2/M checkpoint, but a delayed ATR response contributes to a sustained G2/M checkpoint response. Specifically, upon initiation of the G2/M checkpoint by DNA damage, the DNA end resection results in a switch from ATM dependency to ATR dependency for checkpoint control [48].

Another layer of control for the maintenance of the G2/M checkpoint is the p53 pathway [141] (see Chapter 15). Through transcriptional induction of p21, p53 can indirectly suppress CDK activity, which in turn enables the activation of the pRB tumor-suppressor pathway [33]. The activated pRB reduces the activity of E2F, thereby decreasing the pro-proliferative expression of E2F target genes, such as the APC/C inhibitor Emi1. This promotes the premature activation of APC/C in G2, resulting in the degradation of cyclins A and B and further supporting a G2/M cell–cycle arrest [142].

In summary, in response to DNA damage, mammalian cells can arrest at the G2/M checkpoint due to the inhibition and/ or degradation of CDK1 activators, such as CDC25 and cyclin B combined with the activation of CDK1 inhibitors, such as Wee1. Note that, upon completion of DNA repair, PLK1 is activated, resulting in the phosphorylation-dependent degradation of Wee1 and the activation of CDC25 by nuclear accumulation.

4.4 DNA-Damage Checkpoints and Disease

DNA-damage checkpoints play important roles in diseases including cancer and aging [143]. They influence basic mechanisms of somatic and stem cell physiology, such as renewal, maintenance, and differentiation, but they also help prevent the development of cancer. Specifically, in mammals, at a young age, DNA-damage checkpoints can help to extend lifespan by promoting cancer resistance, while during aging, DNA-damage checkpoints may limit tissue integrity. On the one hand, DNA-damage checkpoints influence the self-renewal, maintenance, and quiescence of somatic mammalian stem cells, with a decreased responsiveness of ATM-p53-dependent checkpoints during aging [143]. On the other hand, the constant activation of DNA-damage checkpoints in the context of increased DNA damage can promote cellular transformation [143]. Likewise, defects in DNA-damage checkpoints can promote tumor progression by abrogating apoptotic and/or senescence programs, as is the case in patients suffering from ataxia telangectasia or Li–Fraumeni syndromes [19,144]. However, although the DDR and DNA damage-associated oncogene-induced senescence are activated in precancerous lesions, their activation in advanced cancers is rarely observed, indicating that malignant cancer cells find ways to bypass DNA-damage checkpoints and senescence [145].

In summary, DNA-damage checkpoints are certainly beneficial at a young age. However, their aging-associated decline associated with the accumulation of DNA damage in tissues has the potential to turn protective responses into damaging responses, possibly resulting in tissue dysfunction and selection of malignant cancer cells.

5. CONCLUSION

Taken together, mammalian cells rely on diverse signal transduction mechanisms to safeguard their genomic integrity. On the one hand, the accurate copying of genetic information in the S phase must be coupled with the precise and equal distribution of chromosomes between daughter cells in mitosis. On the other hand, mechanisms must be in place to detect and repair a broad range of DNA lesions that occur on a regular basis. Therefore, mammalian cells utilize DNA lesionspecific DNA damage-repair pathways to remove unwanted alterations of genetic information. Significantly, the repair of DNA lesions is synchronized with cell-cycle progression by the DDR. In response to DNA damage, a mammalian cell can respond with a transient cell-cycle arrest to allow the repair of DNA damage, or commit to a permanent cell-cycle arrest in the form of senescence, or initiate apoptosis in case the DNA damage is beyond repair. Generally, the DDR protects mammalian cells against the accumulation of DNA lesions which if not removed can cause human diseases including cancer, premature aging, and others. Thus, future research into bettering our understanding of DNA-damage checkpoints and DNA-repair mechanisms in health and disease is very likely to significantly expand our diagnosis, prediction, and treatment options in diverse human diseases.

GLOSSARY

Apoptosis The process of programmed cell death.

Ataxia telangiectasia A rare inherited disorder affecting the nervous system, immune system, and other body systems. Ataxia refers to a poor coordination and telangiectasia to small dilated blood vessels, two hallmarks of the disease.

Cell-cycle checkpoint Specific control mechanisms in eukaryotic cells ensuring a proper cell-cycle progression.

Cellular senescence An irreversible G1 cell-cycle arrest in which cells are refractory to growth factor stimulation.

Cytokinesis The separation of daughter cells by cytoplasmic division at the end of mitosis.

Cytotoxic Any process or agent that kills cells.

DNA damage-induced cell-cycle checkpoints (aka DNA-damage checkpoints) Cell-cycle checkpoints that are specifically activated upon the detection of DNA lesions.

DNA-damage response A complex network of cellular pathways responsible for the detection, signaling and repair of DNA lesions.

E3 ubiquitin ligase An enzyme that catalyzes the transfer of ubiquitin from the E2 ubiquitin-conjugating enzyme to specific protein substrates.

Endogenous DNA lesion A type of DNA damage that is a consequence of endogenous cellular processes.

Exogenous DNA lesion A type of DNA damage that is caused by exogenous genotoxic agents.

Genomic instability (aka genetic or genome instability) Defined as a high frequency of mutations within the genome where mutations can include changes in nucleic acid sequences, chromosomal rearrangements, and/or aneuploidy.

Genotoxic Damaging effects on a cell's genetic material.

Heterochromatin The tightly packed forms of DNA that play roles in the regulation of gene expression.

Li-Fraumeni syndrome A rare cancer predisposition hereditary syndrome associated with p53 mutations.

Malignant transformation The process by which cells acquire the properties of cancer.

Permanent cell-cycle arrest An irreversible exit from cell-cycle progression.

Quiescence The state of a cell when it is not dividing as a consequence of a reversible cell-cycle exit.

Transient cell-cycle arrest A fully reversible exit from cell-cycle progression.

Ubiquitin-mediated degradation E3 ubiquitin ligase-mediated marking of substrates by polyubiquitin chains, which targets substrates for destruction by the proteasome.

LIST OF ACRONYMS AND ABBREVIATIONS

ATM Ataxia telangiectasia mutated ATR ATM and Rad3 related BER Base excision repair CDK Cyclin-dependent kinase CHK1 Checkpoint kinase 1 CHK2 Checkpoint kinase 2 CKI CDK inhibitor **DDR** DNA-damage response **DNA-PK** DNA-dependent protein kinase DSB DNA double-strand break YH2AX H2AX phosphorylated on Ser139 HRR Homologous recombination repair IR Ionizing radiation MK2 MAPK-activated protein kinase 2 MMEJ Microhomology-mediated end joining MMR Mismatch repair MRN MRE11/RAD50/NBS1 complex NER Nucleotide excision repair NHEJ Nonhomologous end joining **PIKK** Phosphatidylinositol 3-kinase-related kinase PLK1 Polo-like kinase 1 pRB Retinoblastoma protein SCF SKP1, Cullin1, F-box protein-containing E3 ligase complex SSA Single-strand annealing SSB DNA single-strand break ssDNA Single-stranded DNA **UV** Ultraviolet

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Authors' Contributions

Valenti Gomez and Alexander Hergovich researched the literature and wrote the manuscript together. Valenti Gomez created all figures. All authors read and approved the final manuscript.

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