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ORIGINAL PAPER

DNA damage checkpoints and cancer

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Abstract DNA damage checkpoint is one of the surveillance systems to maintain genomic integrity. Checkpoint systems sense the DNA damage and execute cell cycle arrest through inhibiting the activity of cell cycle regulators. This pathway is essential for the maintenance of genome stability and prevention of tumor development. Recent studies have showed that the cellular responses towards DNA damage, such as cell cycle arrest, DNA repair, chromatin remodeling, and apoptosis are well coordinated. Here we describe the molecular mechanisms of checkpoint activation in response to DNA damage and the correlation between checkpoint gene mutation and genomic instability.

Keywords Cell cycle · DNA damage · Checkpoint · Cancer

Abbreviations

Mammals

ATM	Ataxia-telangiectasia-mutated
ATR	AT and Rad3-related
NBS	Nimegen breakage syndrome
ATRIP	ATR-interacting protein
MRN	Mre11(Meiotic recombination protein)-Rad50(Radiation-sensitive)-Nbs1(Nijmegen breakage syndrome) complex
Dot1L	Disruptor of telomeric silencing-1 large protein
Tip60	Tat-interactive protein, 60 kDa
BRCA1	Breast cancer susceptibility gene 1
53BP1	p53-binding protein 1
MDC1	Mediator of DNA damage checkpoint protein 1
Gamma-H2AX	Phosphorylated H2AX
DNA-PK	DNA-dependent protein kinase

Fission yeast

Set9	su(var), e (z), trithorax domain protein 9
Crb2	cut5 repeat binding protein 2

Budding yeast

Esa1	Essential SAS2-related acetyltransferase
NuA4	Nucleosomal acetylation of histone H4
Ino80	Inositol 1-phosphate synthase 80
Swr1	Swi2/Snf2-related

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Introduction

In the life of a cell, DNA damage poses a great threat to genome stability, potentially leading to a loss or amplification of chromosome activity, which may result in cellular senescence, cancer or cell death. Among the many types of damage, double strand breaks (DSBs) are the most deleterious to cell survival. To maintain genomic integrity, eukaryotic cells are equipped with coordinated systems to contend with DNA damage, including chromatin remodeling, cell cycle arrest, DNA repair and programmed cell death processes

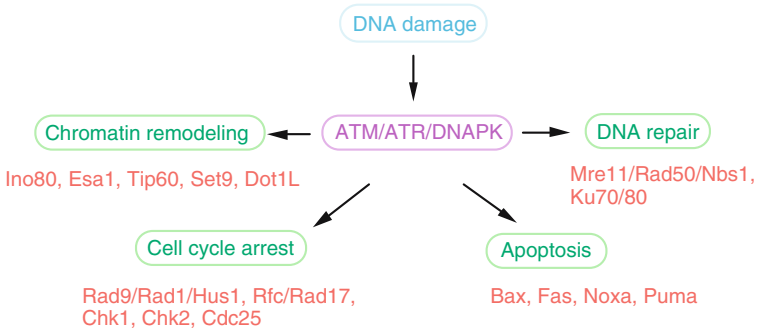

Fig. 1 Schematic model of the cellular response to DNA damage. DNA damage activates PI3KKs, such as ATM, ATR, and DNAPK. The activated PI3KKs transmit the signal to several downstream targets

(Fig. 1). Numerous key players have been identified over the years, but their coordination and interconnectedness in damage control have only recently become evident. Two of these in particular, namely ATM and ATR, function in combination with MRN complexes to recognize damaged DNA and transmit signals to downstream effectors, thereby eliciting several events. These cellular mechanisms are important for determining the potential effects of current cancer therapies in terms of toxicity and efficacy. Recent studies have begun to elucidate the molecular events that lead to cell cycle arrest as coordinated with other responses. In this review, we discuss cellular responses to DNA damage in mammals.

Histone modification and chromatin remodeling

Eukaryotic genomic DNA is packaged with histone and nonhistone proteins into highly condensed chromatin structures. Therefore, repair of DNA damage should include modification and remodeling that would render chromatin more accessible to DNA repair enzymes (Fig. 2). Alteration of the chromatin structure can be achieved by covalent modification of histone tails through ATP-dependent chromatin remodeling (Marmorstein 2001), or by altering the histone composition (Lusser and Kadonaga 2003). Recent studies have shown that modulation of the chromatin structure also plays a role in the DNA repair process (van Attikum and Gasser 2005; Hassa and Hottiger 2005) as well as in transcription and cell cycle checkpoint activation. One of the earliest modifications of chromatin in the damage response is phosphorylation of histone H2AX at Ser139 by members of the PI3 family of kinases, PI3KK, ATM, ATR and DNA-PK (Stiff et al. 2004; Burma et al. 2001; Ward and Chen 2001). Phosphorylated H2AX, referred to as gamma-H2AX, covers a region that may extend up to megabases away from the break sites (Shroff et al. 2004) retaining the regions for mediator proteins such as MDC1 (Goldberg et al. 2003; Lou et al. 2003; Stewart et al. 2003), 53BP1, BRCA1 and the MRN complex (Paull et al. 2000).

Recent studies in yeast have revealed that gamma-H2A functions in the recruitment of histone acetyltransferase, NuA4, and the ATP-dependent chromatin-remodeling complexes, INO80 and SWR1, to a region within two kilobases of a break site (Downs et al. 2004; Morrison et al. 2004; van Attikum et al. 2004; Kobor et al. 2004). The NuA4 complex has histone acetyltransferase activity and mutants of its catalytic subunit, *esa1*, are defective in DNA repair (Bird et al. 2002). INO80 and SWR1 are conserved members of the family of SWI2/SNF2-like ATP-dependent chromatin remodeling complexes and share several subunits with each other. Mutations in subunits of INO80 are less effective in converting double-stranded DNA into single-stranded

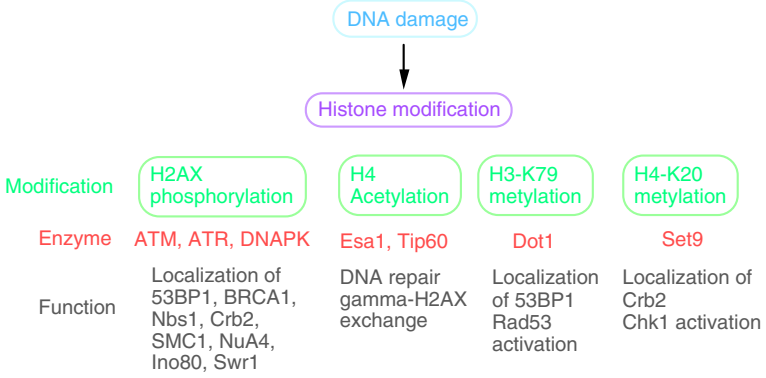

Fig. 2 Histone modification after DNA damage. DNA damage induces several types of histone modifications. These modifications are mediated by distinct enzymes and have unique functions as shown in the figure

DNA at DSB ends, resulting in hypersensitivity to DNA damage (Shen et al. 2000). The SWR1 complex causes chromatin remodeling by exchanging H2A–H2B histone dimers with histone variant Htz1 (referred to as H2AZ in mammals)—H2B dimers in nucleosomes (Mizuguchi et al. 2004). Based on the fact that chromatin remodeling complexes are recruited near the break site, these complexes likely unravel the packed chromatin, allowing repair enzymes to access the DNA. As yet, however, little is known about the changes in histone acetylation at near the break sites that trigger the DNA repair process in mammalian cells. Interestingly, the Tip60 HAT complex, virtually identical to the Esa1 subunit of the yeast NuA4 complex, is also required for DSB repair and exchange of gamma-H2AX. Exogenous expression of Tip60 lacking histone acetylase activity rendered cells defective in double-strand break repair. In Drosophila, Tip60 acetylates the phosphorylated histone variant, H2Av, and replaces it with unmodified H2Av. Therefore, histone modification and remodeling likely facilitates restoration to the undamaged chromatin state.

Histone methylation has also been shown to be linked to the recruitment of checkpoint proteins. Methylation of lysine 79 on histone H3 which is carried out by Dot1L is important for the recruitment of 53BP1 to sites of DNA damage (Huyen et al. 2004). In fission yeast, methylation of lysine 20 on histone H4 is required for recruitment of the 53BP1 homologue Crb2 to sites of damage, allowing full activation of a DNA damage checkpoint (Sanders et al. 2004). Thus, histone modification and chromatin remodeling likely change the higher order chromatin structure and recruit DNA repair and checkpoint proteins to the damage sites.

PI3KK

The kinases PI3KK, ATM, ATR and DNA-PK, initiate kinase cascades that ultimately result in DNA repair, cell cycle checkpoint activation or induction of cell death (Shiloh 2003).

ATM is mainly activated as a damage sensor in response to DNA double-strand breaks. Patients bearing an ATM mutation suffer from a devastating syndrome called ataxia telangiectasia (AT) that causes immunodeficiency, genome instability, clinical radiosensitivity and a predisposition to cancer (Shiloh 1997; McKinnon 2004). Although ATM is not essential for normal cell cycle progression and differentiation, its kinase activity is stimulated by DSBs (Banin et al. 1998). New insights into the molecular mechanism responsible for the initial activation of ATM have recently emerged. Upon DNA damage, ATM is phosphorylated at Ser-1981 and then dissociates from an inactive multimer into active monomers (Bakkenist and Kastan 2003). NBS1 that forms a conserved complex with Mre11 and Rad50 (the MRN complex) associates with ATM, thereby recruiting it to damage sites and enhancing its activity (Lee and Paul 2004). Thus, ATM is likely to be activated through homodimer dissociation via autophosphorylation and recruitment to the site of DNA damage by interaction with NBS1. However, the mechanism involved in ATM autophosphorylation is not yet known in detail. Recently, PP2A has been reported to regulate ATM autophosphorylation (Goodarzi et al. 2004). In the absence of DNA damage, ATM associates constitutively with PP2A. DNA damage causes a rapid dissociation of the ATM–PP2A complex, leading to its autophosphorylation. In addition, ATM is acetylated by Tip60 histone acetylase and this modification is important for ATM kinase activity (Sun et al. 2005).

ATR was discovered from its sequence similarity to ATM and Rad3 (Cimprich et al. 1996) and was shown to play an essential role in DNA damage repair and DNA replication checkpoint activation (Abraham 2001). Mutations in ATR have been reported in a subset of patients with Seckel syndrome, which is a human autosomal recessive disorder (O'Driscoll et al. 2003). Mice lacking ATR succumb to early embryonic death, indicating that ATR is essential for cell viability (Brown and Baltimore 2000; de Klein et al. 2000). ATR regulates the timing of DNA replication origin firing (Shechter et al. 2004) and initiation of mitotic events on centrosomes through phosphorylation of Chk1 (Kramer et al. 2004). Although ATR kinase activity appears not to be stimulated by damaged DNA or an inappropriate replication fork, its subcellular localization is likely to be regulated by DNA damage or replication blocks. As a result of processing damaged lesions or in replication fork stalling, ATR forms a heterodimer with ATRIP that binds to UV damaged DNA or to RPA-coated ssDNA (Zou and Elledge 2003), and the affinity is higher than to undamaged double-stranded DNA. In in vitro experiments, RPA was shown to stimulate the binding of ATRIP to ssDNA (Zou and Elledge 2003). Therefore, the active ATR kinase is localized to the ssDNA region through the interaction of ATRIP and RPA, leading to phosphorylation of critical substrates such as Rad17 and Chk1.

ATR is thought to be unresponsive to DNA double-stranded breaks, however, it plays a role in the response to IR-induced DNA damage. Irradiation

induces the formation of RPA-coated ssDNA generated by nuclease resection or stalled replication forks caused by unrepaired SSB/DSB, and this triggers ATR activation. Consistent with this observation, ATM regulates the recruitment of ATR to sites of DNA damage, leading to DSB-induced Chk1 phosphorylation (Jazayeri et al. 2005).

DNA-PK is also a member of the PI3KK family, and is composed of a catalytic subunit termed DNA-PKcs and DNA-binding Ku heterodimer consisting of Ku70/80 subunits (Lee and Kim 2002). DNA-PK is activated by DNA damage, recruited rapidly to DSBs and phosphorylated at multiple sites. Mice deficient in functional DNA-PKcs show hypersensitivity to IR, undergo accelerated aging, harbor shorter telomeres and have defective NHEJ (Espejel et al. 2004).

The principal step in activation of these PI3KKs is considered to be their recruitment to damage sites. A conserved motif was identified in C-terminal regions of NBS1, ATRIP and Ku80, which are required for interaction with ATM, ATR and DNA-PKcs respectively, and common mechanisms involving this motif allow these PI3KK partners to function in the recruitment of PI3KKs to the damage sites (Falck et al. 2005).

Once the activated PI3KKs are recruited to the DNA break sites, they can then phosphorylate their critical targets. In the case of ATM, the substrates are NBS1 (Lim et al. 2000; Zhao et al. 2000; Gatei et al. 2000; Wu et al. 2000), BRCA1, SMC1 (Kim et al. 2002; Yazdi et al. 2002), MDC1 (Goldberg et al. 2003; Lou et al. 2003; Stewart et al. 2003), 53BP1 (DiTullio et al. 2002), Chk2 (McGowan 2002; Bartek et al. 2001), p53 (Banin et al. 1998; Canman et al. 1998; Khanna et al. 1998). Mammalian cells lacking any of these genes show decreased viability and impairment at cell cycle checkpoints. ATR phosphorylates Rad17 (Zou et al. 2002), ATRIP (Cortez et al. 2001), Chk1 (Zhao and Piwnica-Worms 2001; Guo et al. 2000), and p53 (Tibbetts et al. 1999). Of the many potential substrates of DNA-PKs reported, phosphorylation of WRN (Werner protein) appears to be physiologically important (Karmakar et al. 2002).

Checkpoint signaling

G1/S checkpoint

In the presence of DNA damage, the G1/S checkpoint prevents replication of damaged DNA through two distinct signal transduction pathways (Fig. 3). One involves the degradation of Cdc25A phosphatase that induces rapid G1/S arrest. Chk2 and Chk1 activated by ATM and ATR phosphorylate Cdc25A, which is in turn degraded by the ubiquitin proteasome pathway (Mailand et al. 2000; Falck et al. 2001). Degradation of Cdc25A results in the inactivation of Cdk2 and prevents Cdc45 from loading onto chromatin (Arata et al. 2000). Because Cdc45 is essential for the recruitment of DNA polymerase alpha, lack of Cdc45 in corporation into the chromatin structure inhibits new origin firing. This pathway appears to play a role in the initial G1/S checkpoint arrest. In order to maintain this arrest, transcriptional responses are mediated by p53, which is the most frequently mutated tumor suppressor gene in human cancers (Hollstein et al. 1991; Hickman et al. 2002; Michael and Oren 2002). Phosphorylation of p53 on Ser15 by ATM/ATR and on Ser20 by Chk1/Chk2 inhibits its nuclear export and degradation, resulting in the accumulation of p53 in the nucleus. The ubiquitin ligase Mdm2 binds to p53 and promotes its ubiquitination and degradation, thereby maintaining a low level of p53 protein. ATM also phosphorylates Mdm2 on Ser395 and decreases the possibility of an interaction between Mdm2 and p53, which results in a p53 accumulation (Khosravi et al. 1999; Maya et al. 2001). Chk2 likely helps to stabilize p53 protein after DNA damage, although there have been conflicting (Hirao et al. 2000; Jack et al. 2002; Takai et al. 2002; Ahn et al. 2003; Jallepalli et al. 2003). The key transcriptional target of p53 is Cdk inhibitor p21, which inactivates G1/S-promoting cyclinE/Cdk2 kinase. p21 also prevents entry into S phase by RB-mediated sequestration of transcription factor E2F, thereby inducing a variety of genes which are required for entry into S phase (Bartek and Lukas 2001; Lin et al. 2001).

Intra-S checkpoint

During the S-phase, damaged DNA inhibits replicative DNA synthesis, which is referred to as an intra S-checkpoint. The intra-S checkpoint is regulated by two distinct pathways, namely ATM/ATR–Chk1/Chk2–CDC25A and ATM–NBS1–SMC1 (Falck et al. 2002). Depending on the type of DNA damage, ATM or ATR phosphorylates Chk2 or Chk1, respectively, resulting in the phosphorylation and degradation of Cdc25A (Falck et al. 2002; Zhao et al. 2002; Sorensen et al. 2003). Downregulation of Cdc25A subsequently causes inactivation of the S-phase-promoting cyclin E/Cdk2 and prevents loading of Cdc45 on replication origins. The phosphorylation of Nbs1 on S343 by ATM is required for activation of the Nbs1–Mre11–Rad50 complex and the intra-S checkpoint (Lim et al.

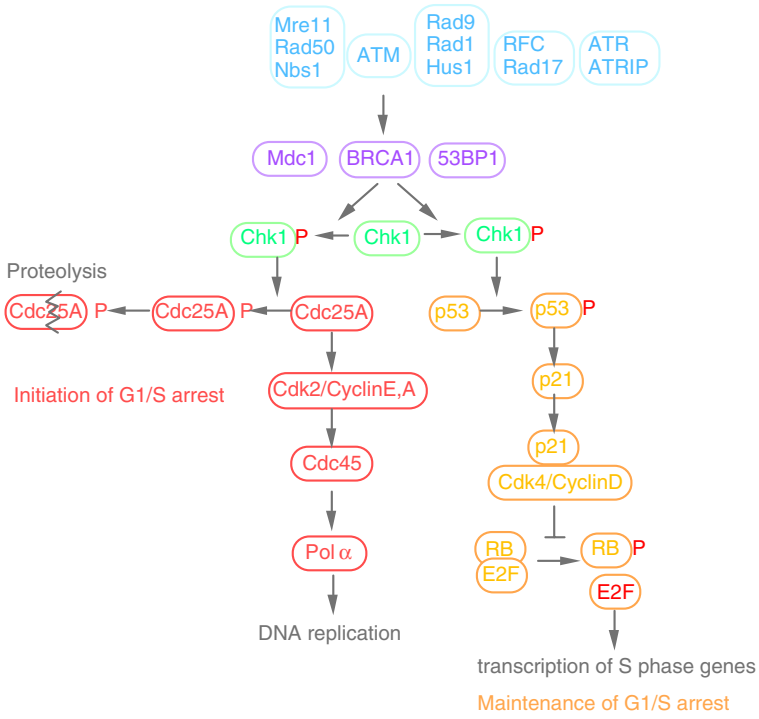

Fig. 3 Schematic model of checkpoint signaling pathways at G1/S in response to DNA damage. DNA damage activates ATM and ATR. The activated ATM and ATR then phosphorylate mediator proteins including MDC1, BRCA1, and 53BP1. Collaboration of the activated ATM and ATR, and the phosphorylated mediators activate Chk1. The activated Chk1 ultimately transmits signals via two distinct pathways as shown in the figure

2000; Zhao et al. 2000). Depending on the phosphorylation state of Nbs1, one of the components of the cohesion complex, SMC1, is phosphorylated on Ser-957 and Ser-966 by ATM and this phosphorylation is required for the intra-S checkpoint (Kim et al. 2002; Yazdi et al. 2002). Mediator-adaptor proteins, such as 53BP1, BRCA1, and MDC1, also contribute to the intra-S checkpoint by regulating the phosphorylation of downstream proteins such as Chk1, Chk2, and NBS1.

G2/M checkpoint

The G2/M checkpoint prevents cells from entry into mitosis through the inhibition of cyclinB/Cdc2 kinase by Chk1/Chk2, p38-mediated subcellular sequestration, degradation, and inhibition of the Cdc25 family of phosphatases (Fig. 4). The initiation of G2/M arrest is also achieved independently with p53. Following DNA damage, the ATM–Chk2–Cdc25A and/or the ATR–Chk1–Cdc25A pathways are activated. BRCT motif

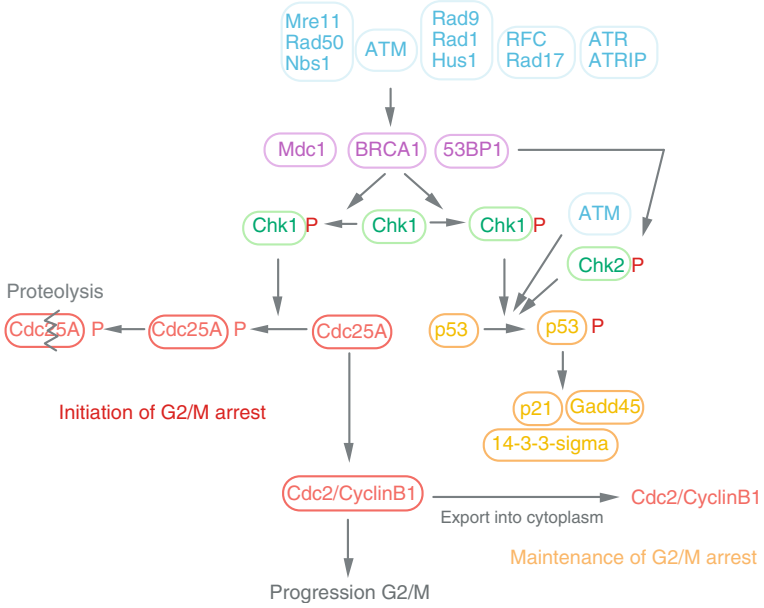

Fig. 4 Schematic model of checkpoint signaling at G2/M upon DNA damage. Activated Chk1 phosphorylates Cdc25A. The phosphorylated Cdc25A is then degradated through the ubiquitin-proteasome pathway, resulting in the inhibition of Cdc2/cyclin B. The activated Chk1 also phosphorylates and stabilizes p53, leading to the induction of several downstream target genes including p21, Gadd45, and 14-3-3 sigma

proteins, such as 53BP1, MDC1, BRCA1, play roles in the activation of Chk1 and Chk2. Phosphorylated Cdc25A leads to its degradation and subsequent inactivation of cyclinB/Cdc2. The roles of WEE1 and MYT1 kinases in the mammalian G2/M damage checkpoint control are not yet clear, but it is known that p53-dependent mechanisms are important for the maintenance of G2 arrest. The critical targets of p53 are the Cdk inhibitor p21, GADD45 that causes the dissociation of the Cdc2 and cyclin complex and 14-3-3 sigma, which sequesters the cyclinB/Cdc2 complex in the cytoplasm (Chan et al. 1999). Cells lacking these genes exhibit a G2/M checkpoint defect. In addition, p53 represses the transcription of cdc2 and cyclinB. Two isoforms of MAP kinase, p38 alpha and p38 gamma, are also implicated in the G2/M damage checkpoint through the regulation of Cdc25B and Chk2, respectively (Wang et al. 2000; Bulavin et al. 2001)

Conclusions

The coordinated activation of cell cycle checkpoints, DNA repair and apoptosis are essential for the maintenance of genome integrity and tumor suppression. Given that mutations or decreased expression of the genes implicated in checkpoint control are detected in the most of cancers, proper checkpoint signaling is essential for preventing cancer. Mutations in Chk1 or Chk2 have been reported in sporadic or familial cancers (Bell et al. 1999). Since abrogation of the G2 checkpoint might be more detrimental in cancer cells lacking p53 than in normal cells, new anticancer drugs targeting the G2 checkpoint inhibitor appear to be important for the development of therapies with fewer side effects. Currently, Chk1 and Chk2 are considered potential targets. An approach that combines conventional anticancer treatments such as radiation and chemotherapy with the use of new small molecule inhibitors of Chk1 and Chk2 should prove to be effective in eliminating cancer cells.

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