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Damage and Replication Stress Responses

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1. Introduction

The character of DNA replication is high fidelity. Precise and complete DNA replication is critical for the maintenance of genetic stability. Failures in these processes are major sources of genomic instability and will lead to cancer or other diseases. A wide variety of factors, such as DNA replication errors, spontaneous chemical reactions, reactive metabolic products, exogenous environmental agents or some anticancer therapeutics e.g. 5'-aza-2'-deoxycytidine (5-Aza-CdR), can cause DNA damage (Wang et al, 2008; Zhu et al, 2004; Chai et al, 2008; Hoeijmakers, 2009). It is estimated that DNA damage occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day (Lodish, 2004). In order to deal with problems under which the genome is vulnerable to injury or replication stress, eukaryotic cells elaborate a genomic maintenance apparatus, which is termed the DNA damage response (DDR) and replication stress response, including various checkpoint, signal-transduction and effectors system, which monitor problems and trigger a comprehensive cellular response pathway to prevent genome integrity. The extent of DNA damage depends on the type of environment to which it is exposed (Hoeijmakers, 2001). So, organisms must be capable of recognizing and dealing with each type of damage. It is not surprising that there are various different types of DNA damage response and repair systems.

ATM and ATR are at the top of the DNA damage pathways. Although a cross-talk exists between the ATM and ATR pathways, ATM primarily seems to be involved in the detection of DNA double-strand breaks via Mre11/Rad50/Nbs1 complex (MRN), ATR is critical for cellular responses to a variety of DNA damage and stalled replication forks (Hefferin & Tomkinson, 2005). When these protein kinases activated, they eventually phosphorylate and modulate the downstream effectors (e.g., Chk1 and Chk2) and multiple additional substrates that initiate the cellular responses.

Dynamic changes in protein post-translational modifications play a significant role in most cellular signalling pathways. More and more proteins were found in a variety of post-translational modifications in response to DNA damage and genotoxic stress, such as phosphorylation, acetylation, sumolyation, methylation and ubiquitylation. Recent studies indicate that a crosstalk between multiple protein modifications exists, which collaboratively regulates signal transduction of DNA damage and genetic stresses.

Actually, the DNA damage and replication stress response consists of multiple interconnected pathways, which impact the cell cycle, DNA replication, DNA repair, transcriptional regulation, chromatin remodelling, metabolic and other cellular biological processes (Rouse & Jackson, 2002; Zhou & Elledge, 2000). In this chapter, we focus on recent findings of DNA damage response signalling pathways.

2. The DNA damage response pathways

The ability of cells to respond to DNA damage and replication stress response is critical for cellular survival. The evidence indicates that DNA damage and replication stress response are a cascade signal transductional process, which consists of multiple interconnected pathways through which sense damage or replication stress, transduce the damage signals, and trigger cellular responses, including cell cycle arrest, DNA repair or apoptosis (Shiloh, 2003; Bakkenist & Kastan, 2004; McGowan & Russell, 2004). In mammalian cells, PI3K family members, ATM and ATM-Rad3-related (ATR) are central to the entire DNA damage response (Elledge, 1996). All types of DNA lesions induce responses to these two main signalling pathways. Next, we will summarize the model of cells dealing with DNA damage and replication stress through these two pathways.

2.1 ATM dependent cellular response to DNA double strand breaks pathway

The DNA double strand breaks (DSBs) are the most dangerous damage type for the organisms because they are prone to cause genomic rearrangements, cancer predisposition, and cell death if not repaired correctly (Wyman & Kanaar, 2006). Many endogenous and exogenous factors may induce DSBs, such as IR, UV, reactive oxygen species (ROS) or topoisomerases inhibitors (Tanaka, 2006; Tanaka, 2007). Cellular responses to DSBs, include complex signal-transduction, cell-cycle-checkpoint and repair pathways, play a pivotal role in maintenance of the genome integrity. It is accepted that ATM is a central component of the DSB signalling cascade (Khanna & Jackson, 2001; Shiloh, 2001; Abraham, 2001).

2.1.1 ATM

ATM is the gene product mutated in ataxia telangiectasia discovered in 1995 and characterized by progressive cerebellar ataxia, immune deficiencies, radiation sensitivity, and an increased risk of cancer (Lavin & Shiloh, 1997; Savitsky et al, 1995). ATM is a serine-threonine kinase which belongs to the phosphatidylinositol-3-kinase (PI3K) like protein kinases (PIKK) family. In normal condition, ATM exists in an inactive form of dimer or multimer. Following DSBs, ATM was dissociated into an active monomer through autophosphorylation (Bakkenist & Kastan, 2003). Upon activation, ATM is recruited to DNA breaks where it initiates phosphorylation of several substrates such as p53, Mdm2, BRCA1, Chk2 and Nbs1 to initiate cell cycle arrest, DNA repair, and apoptosis (Lukas et al., 2003; Shiloh Y, 2006).

2.1.2 Mechanism for the activation of ATM

Many progresses have been made on understanding how DSBs activates ATM. Several investigations suggest that the Mre11-Rad50-Nbs1 (MRN) complex is involved in ATM activation and recruitment to the sites of DSBs (Uziel et al, 2003; Cerosaletti & Concannon, 2004), because attenuated activation and no recruitment of ATM to DSBs upon damage were found in Mre11- and Nbs1- deficient cell lines. Earlier studies have shown that MRN lies

downstream of the ATM mediated DNA damage signalling pathway because ATM can phosphorylate the components of the MRN complex in response to IR (Lim & Ki, 2000; Wu & Ranganathan, 2000; Zhao & Weng, 2000). However, further analyses demonstrate that the MRN complex is more like an upper actor of ATM pathway (Uziel et al, 2003；Difilippantonio et al, 2005; Carson et al, 2003). Because the MRN complex was reported to play a role in early detection of DSBs which initiates the localization of ATM to DSBs (Lee & Paull, 2004, 2005). It is now established that ATM at DSBs is a spatio-temporal dynamics mechanism. At first, change in chromatin structure caused by DNA DSB partially activates ATM (Berkovich et al, 2007). Activated ATM rapidly phosphorylates H2AX on its C-terminus, and γ-H2AX subsequently recruits MDC1 (mediator of DNA damage checkpoint protein 1) to bind to it and acts as a scaffold, in turn, recruits MRN at the flanking chromatin of DSBs (Burma et al, 2001; Stucki & Jackson, 2006; Lou et al, 2006; Stucki et al, 2005), which promotes accumulation of ATM to sites of DSBs, where it is fully activated (Lavin, 2008). In addition, MDC1 also mediates the interaction between ATM and γ-H2AX, which contributes to the extended phosphorylation of H2AX and the maintenance of the DSB response (Huen & Chen J, 2008).
Autophosphorylation has been proposed as the other mechanism for ATM activation. Three phosphatases, PP2A, PP5 and WIP1, have been reported to be involved in the control of ATM activation. Autophosphorylation on Ser367, Ser1893, Ser1981 and a new site S2996 are present on activated ATM through dissociation of the inactive dimeric ATM to an active monomeric form (Bakkenist & Kastan, 2003; Kozlov et al, 2010). Recent reports indicate that there is a Nbs1-independent ATM activation pathway which regulates ATM activity through its effect on ATM autophosphorylation (Kanu & Behrens, 2007; Sun et al., 2005, Gupta et al., 2005; Richard et al, 2008). Interestingly, notwithstanding the difference on the importance of ATM autophosphorylation in humans and in mice, this is certainly the case in human cells that autophosphorylation of ATM at serine 1981 is required for the interaction of ATM with MDC1, which stabilizes ATM at DSBs and thereby promotes a full-scale response to DNA damage (Sairei et al, 2009). Once activated, ATM directly or indirectly phosphorylates approximately 30 substrates, such as Chk2, p53, BRCA1, RPAp34, H2AX, SMC1, HDMX, FANCD2, Rad17, Artemis or Nbs1, which are involved in cell cycle checkpoint control, apoptotic responses and DNA repair.

2.2 “two-man rule” of ATR in response to DNA damage

Like ATM, ATR (ATM- and Rad3-related) is a nuclear Ser/Thr kinase which belongs to the PIKK family (Bentley et al, 1996). ATR forms a stable heterodimer with its interacting partner ATRIP which can be activated by DNA damage (Cliby et al, 1998; Wright et al, 1998). Compared with the ATM, ATR can respond to a broader spectrum of genotoxic stimuli including DNA replication inhibitors (such as hydroxyurea), UV radiation, ionizing radiation, and agents that induce DNA interstrand cross-links and generate single-stranded DNA (Wright et al, 1998; Yang et al, 2003; Costanzo & Gautier, 2003; Wang et al, 2008). Once the break occurs, ATR is recruited by ATR-IP to the sites of DNA damage and interacts with RPA to initiate the response (Zou & Elledge, 2003b; Cortez et al, 2001; Wang et al, 2008).

2.2.1 Mechanisms of ATR activation

ATR is involved in many different types of DNA damage responses. The common feature is that ATR is activated by single strand DNA (ssDNA), which is a common intermediate structure that can be formed at sites of DNA damage and replication stress, or induced by

most cancer chemotherapies (Costanzo & Gautier, 2003; Zou & Elledge, 2003b). A study shows that both ssDNA and a 5′ junction are sufficient to activate ATR signalling (MacDougall et al, 2007). ATR activation requires assembly of a protein complex on ssDNA, which begins with ATR-ATRIP complex loading on the RPA-coated ssDNA (Stokes et al, 2002; MacDougall et al, 2007; Byun et al, 2005). Earlier works have shown that RPA binds to ssDNA and then recruits ATR-ATRIP by interacting with ATRIP (Cortez et al, 2001; Ball et al, 2007). However, the recruitment to ssDNA is not sufficient for ATR activation (Ball et al., 2005; Namiki & Zou, 2006; Yoshioka et al., 2006), it requires additional ATR regulator, Rad9-Rad1-Hus1 (9-1-1) complex, a heterotrimeric ring-shaped structure like PCNA (Parrilla-Castellar et al., 2004). The 9-1-1 complex recognizes a DNA end that is adjacent to a stretch of RPA-coated ssDNA through working with RFC-RAD17 (Ellison & Stillman, 2003; Zou et al., 2003a; Bermudez et al., 2003). Current models for ATR activation suggest that the 9-1-1 mediated recruitment of TopBP1 to the ATR-ATRIP complex, and the ATR-activating domain of TopBP1 activates the kinase activity of ATR (Harper & Elledge, 2007; Cimprich & Cortez, 2008; Shiotani & Zou, 2009; Yan & Michael, 2009). In addition to be an activator of ATR, TopBP1 is also a substrate of ATR. The phosphorylation of TopBP1 on residue S1131 by ATM enhanced the interaction of it with ATR-ATRIP, which suggests that TopBP1 promotes a feed-forward signalling loop to amplify ATR-mediated signals (Yoo et al., 2007). Thus, sustained co-localization of the ATR-ATRIP and 9-1-1-TopBP1 complexes at the DNA damage site may increase their local concentration so that ATR activation is stimulated continually by TopBP1. However, recruitment of the ATR-ATRIP and 9-1-1-TopBP1 complexes to sites of DNA damage or stalled replication forks is independent events (Bonilla et al, 2008; Kondo et al, 2001; Melo et al, 2001; Zou et al, 2002; You et al, 2002; Lee et al, 2003). Therefore, there is the two-man rule in TopBP1-dependent regulation of ATR activity, by which ATRIP and 9-1-1 together control the TopBP1 to initiate ATR signalling.

As described above, activation of ATM involves its autophosphorylation, which helps it convert an inactive dimmer form into an active monomers form. Some phosphorylation sites on ATR and ATRIP have been found (Cimprich & Cortez, 2008), unlike ATM, as yet, none of these identified modifications has been reported to contribute to ATR activation and the oligomerization status of ATR-ATRIP.

2.3 Interplay between ATM and ATR pathway

It was previously thought that ATM and ATR had overlapping but distinct roles in response to DNA damage. However, a current study demonstrate a high degree of cross-talk and connectivity. For instance, ATM and ATR collaborate in the IR-induced G2/M checkpoint, but incomplete DNA replication in mammalian cells can prevent M phase entry independent of ATR (Brown & Baltimore, 2003). Recently, Trenz et al indicate that both ATM and ATR promote Mre11-dependent restart of collapsed replication forks and prevent accumulation of DNA DSBs (Trenz et al, 2006). Another study showed that ATR is activated rapidly by IR, and both ATM and Mre11 enhance ATR signalling (Myers & Cortez, 2006). The new data demonstrate that ATR is required for the response to either replication stress or IR without any role for ATM (Paul et al. 2004).

3. DNA damage response pathways and cell cycle checkpoints

The maintenance of genome stability is critical to the survival and propagation of all cellular organisms. The cell cycle is required for cell growth and cell division into two daughter

cells. Cell cycle checkpoints are regulatory pathways that control the cell cycle events in the right order. DNA is vulnerable to diverse types of injury throughout the cell cycle. In response to DNA damage, checkpoint surveillance mechanisms initiate a cascade of events which coordinate cell cycle arrest and facilitate DNA repair pathways. These checkpoints include the G1/S, intra-S and G2/M of the cell cycle and are controlled by the ATM/Chk2 and ATR/Chk1 pathways. We will discuss the progresses of different signalling pathways involved in different checkpoints.

3.1 G1 Checkpoint

G1 checkpoint is the first checkpoint making the key decision of whether the cell should divide or arrest, which prevents the damaged DNA from being replication (Bartek & Lukas, 2001). The major player in the G1 checkpoint is the p53 protein. In normal cells, p53 is maintained at low levels due to interaction with MDM2, which targets p53 for degradation in the cytoplasm (Alarcon-Vargas & Ronai, 2002). In response to distinct or partially overlapping types of DNA damage, p53 is activated by ATM or ATR through phosphorylating different Ser/Thr residues directly and indirectly (Matsuoka et al., 2000; Maya et al., 2001; Shieh et al., 2000). The phosphorylation of Ser15 appears important in enhancing p53 transcriptional transactivation activity (Dumaz & Meek, 1999; Wang et al, 2008). The result of p53 activation is the up-regulation of various target genes (such as MDM2, GADD45a, and $p21^{Waf1/Cip1}$), some of which are involved in the DNA damage response. $p21^{Waf1/Cip1}$ elicits G1 arrest through suppressing Cyclin E/Cdk2 kinase activity (Bartek & Lukas, 2001). In other p53 target genes, such as Gadd45 and BIG2, also lead to G1 arrest. p53 lead to G1 checkpoint arrest in multiple pathways, now, p53 is reported to contribute to maintain G1 checkpoint control via activating microRNAs directly.

3.2 S-phase Checkpoint

The S-phase checkpoint monitors cell cycle process and lowers the rate of DNA replication after DNA damage. ATM plays a primary role in contributing to S-phase checkpoints although it overlapping with the ATR dependent pathway in maintenance of the S-phase checkpoint. In response to ionizing radiation, ATM phosphorylates Nbs1 and Chk2 and triggers two parallel cascades of the DNA damage responses to activate the S-phase checkpoint. One is the ATM–Chk2–Cdc25A pathway; the other is the ATM dependent NBS1/BRCA1/SMC1 pathway, though the mechanism of this pathway is not well understood (Falck et al., 2002). On the other hand, the ATR-Chk1 pathway is also involved in the S-phase checkpoint arrest auxiliary in response to IR. Furthermore, the ATR-Chk1 pathway plays a dominant role in directing S-phase checkpoint arrest in response to UV damage and replication errors (Abraham, 2001).

3.3 G2 Checkpoint

The G2 cell cycle checkpoint is an important control point which functions to prevent damaged DNA from being segregated into daughter cells. This checkpoint activation depends on the maintenance of Cdc2 phosphorylation on T14 and Y15 (Rhind et al., 1997). ATM and ATR both indirectly modulate the phosphorylation status of these sites in response to DNA damage. Different from other checkpoints, ATR mainly controls the response to UV damage and replication blocks. The response to IR is also mediated primarily by ATR while ATM plays a supporting role（Graves et al, 2000). Upon DNA

damage, ATR and ATM phosphorylate their downstream kinases Chk1 and Chk2, respectively, and then phosphorylate the phosphatase Cdc25C on Ser216 (Peng et al., 1997). The phosphorylated Cdc25C binds with 14-3-3 protein and is sequestered in the cytoplasm, which prevents Cdc25C from dephosphorylating Cdc2 in the nucleus and the cells remain arrested in the G2 phase (Lopez-Girona et al., 1999; Peng et al., 1997).
p53 also plays a role in the G2/M checkpoint (Passalaris et al., 1999). Activated p53 in response to DNA damage results in G2/M checkpoint arrest through induction of GADD45 (Zhan et al., 1994). In addition, p53-dependent transcriptional repression of cdc2 and cyclin B may also contribute to the G2/M checkpoint (Passalaris et al., 1999).

4. DNA damage response and protein post-modifications

Post-translational modifications play a vital role in harmonizing cellular response to DNA damage. More and more proteins were found occurring in a variety of post-translational modifications including phosphorylation, acetylation, methylation and ubiquitylation in response to DNA damage or genotoxic stress. Recent research suggests that a crosstalk exists between multiple protein modifications. Here, we will summarize recent findings of protein post-translational modifications in coordinating the DNA damage response signalling cascade.

4.1 Protein phosphorylation modification in response to DNA damage

Signal transduction is predominantly mediated by a cascade of protein phosphorylation and dephosphorylation reactions, which is of prime importance for the organisms to sense the external and internal stimuli and generate the appropriate responses. Protein phosphorylation plays the same role in cellular DNA damage response. As indicated above, in responding to DSB signalling, ATM undergoes autophosphorylation, which seems to be instrumental in the monomerization and activation of ATM. It seems that DNA lesions activate various protein kinases, such as ATM and ATR, which transduce the damage signalling by directly phosphorylating or mediating the phosphorylation and activation of numerous substrates involved in the DNA repair machinery, the cell cycle checkpoints and apoptosis (Abraham, 2001; Osborn et al., 2002). So far, more than 700 proteins have been identified to be phosphorylated in response to DNA damage (Matsuoka et al., 2007). A signalling cascade is initiated starting with the phosphorylation of H2AX (γ-H2AX). γ-H2AX is a chromatin-based signal that regulates the assembly of DNA damage response proteins at the break sites and induction of DNA repairs (Lavin, 2008; Cook et al., 2009). So, the H2AX phosphorylation level is not only important as a marker of the DNA damage response, but also involve in DNA repair processes (van Attikum & Gasser, 2009).
The effector p53 stands at the cross-roads of cellular responses to various stresses (Appella & Anderson, 2001; Bode & Dong, 2004). DNA damage leads to specific phosphorylation modifications of p53 protein. Up to date, at least 20 phosphorylation sites have been detected in the p53 molecule in human cells following DNA damage (Bode & Dong, 2004). Some of which were phosphorylated by ATM in response to irradiation and chemotherapeutic drugs (Banin et al, 1998; Canman et al, 1998), whereas some are phosphorylated by ATR when cells are treated with UV or anti-cancer drugs (Appella & Anderson, 2001; Wang, 2008). Phosphorylation of p53 usually modulates its stability and sequence-specific DNA binding activity (Bode & Dong, 2004). Two major phospho-binding modules, the BRCA1 C-terminal repeat (BRCT) and the forkhead-associated (FHA) domain,

which are present in many proteins are involved in the cellular response to DNA damage, and facilitated protein-protein interactions in the recruitment and activation of damage signalling (Yu et al, 2003; Hofmann & Bucher, 1995; Li et al, 2002).

4.2 Protein acetylation in response to DNA damage

Phosphorylation is not the only post-translational modification in cellular response to DNA damage. For instance, following DSB, ATM is activated in the vicinity of the break and is recruited to the break site by the MRN complex where it is fully activated, facilitated by not only autophosphorylation but also acetylation of ATM (Bakkenist & Kastan, 2003; Sun et al, 2007). The study showed that after DNA damage, CK2 phosphorylates and releases HP1β from chromatin which recruits a ATM-Tip60 complex to MRN at the break site. This promotes interaction between Tip60 acetyltransferase and the unbound histone H3 K9me3 leading to acetylation and activation of ATM (Sun et al, 2009). A single site at Lys3016 is acetylated by Tip60 acetyltransferase. This mutation inhibits the monomerization and up-regulation of ATM activation by DNA damage, further prevents ATM-dependent phosphorylation of p53 and checkpoint kinase-2 (Chk2) (Sun et al, 2005, 2007).

As the recruitment of the Tip60 acetyltransferase, the deacetylase enzymes HDAC1, HDAC2, HDAC4, SIRT1, and SIRT6 also have been observed at DSB sites in mammalian cells (Kao et al. 2003; Oberdoerffer et al. 2008; Kaidi et al. 2010; Miller et al. 2010). For instance, the MRN complex serves as a sensor for the detection of DSBs and is involves in the S phase checkpoint (Paull & Lee, 2005; van den Bosch et al, 2003). The acetylation level of NBS1 was recently reported to be tightly regulated by deacetylase SIRT1 (Yuan et al., 2007). Moreover, SIRT6-dependent deacetylation of the CtIP in response to DSBs stimulates the RPA and RAD51 foci, thus promoting ATR signalling and DSB repair (Kaidi et al. 2010). Furthermore, histone acetylation can regulate the dynamics of DDR factors in the vicinity of DNA breaks.

p53 acetylation also plays important roles in response to various types of DNA damage (Gu & Roeder, 1997; Lill et al, 1997; Nag et al, 2007). Transcription factors with histone acetyltransferase activity, p300/CBP, p300/CBP-associated factor (PCAF), and Tip60 are reported to be mainly responsible for the p53 acetylation (Liu et al., 1999; Sykes et al.,2006; Tang et al., 2006, 2008). p53 acetylation can increase its sequence-specific DNA binding capacity (Gu & Roeder, 1997; Zhao et al, 2006; Luo et al, 2004) or enhance its stabilization by inhibiting ubiquitination of p53 mediated by MDM2 (Li et al, 2002; Ito et al, 2002). Recently our studies indicate that histone deacetylase inhibitors and other chemical agents also induce p53 acetylation through the DNA damage response pathway (Zhao et al, 2006; Wang et al, 2008). Novel discoveries further confirm that p53 acetylation is an indispensable event for mediating the p53 response (Kruse & Gu, 2009). However, the regulatory mechanisms involving in this posttranslational modification are still largely unknown.

4.3 Protein ubiquitylation in response to DNA damage

Ubiquitylation is the process by which the 76-amino-acid polypeptide ubiquitin is attached to the target protein singly (monoubiquitylation) or in the form of polyubiquitin chains (polyubiquitylation) via the covalent bond. This is an enzyme cascade reaction which is involved by ubiquitin E1, E2 and E3 ligase proteins (Pickart, 2001). A growing number evidences have shown that ubiquitylation and deubiquitylation are important regulatory mechanisms in response to DNA damage and genotoxic stresses. Assembly of DNA damage response proteins at the break site is catalyzed by the E3 ubiquitin ligases.

Consistent with these actions, several ubiquitin ligases have been shown to accumulate at sites of DNA breaks in mammalian cells, including BRCA1, RNF8, RNF168, RAD18, HERC2, and PRC1 (Polycomb-repressive complex 1) (Scully et al. 1997; Kolas et al. 2007; Doil et al. 2009; Huang et al. 2009; Stewart et al. 2009; Bekker-Jensen & Mailand, 2010; Chou et al., 2010; Lavin, 2008). For example, phosphorylation of MDC1 at ATM consensus sites promotes interaction with RNF8, the E3 ubiquitin ligase, which in turn ubiquitylates H2A, leading to the accumulation of 53BPI, BRCA1, and other proteins at the site of damage (Kolas et al, 2007; Mailand et al, 2007). Ubiquitylated H2A serves as an interacting partner for RNF168 that further propagates the ubiquitylation of H2A and other unknown targets at the double-strand break site (Doil et al, 2009; Stewart, 2009). In addition, FANCD2 (Fanconi anemia complementation group D2) is monoubiquitinated during the S phase (Taniguchi et al. 2002) and in response to various DNA damaging agents, which is required for its localization to DNA damage foci (Garcia-Higuera et al. 2001). It is demonstrated that ATR is required for efficient FANCD2 monoubiquitination and foci assembly in response to various genotoxic stresses, including IR and MMC. Another example is p53, which is kept at low level in unstressed cells through Mdm2-mediated polyubiquitination, which leads to nuclear export of p53 and subsequent proteasomal degradation. DNA damage attenuates polyubiquitination of p53, thereby stabilizes and activates p53 as a transcription factor, up-regulating expression of numerous proteins involved in cell cycle control, apoptosis and senescence (Toledo & Wahl, 2006; Bode & Dong, 2004).

Besides ubiquitylation, deubiquitylation has also been documented as an opposite way to regulate protein stability in response to genotoxic stress. A number of de-ubiquitylating enzymes (DUBs) were identified at double-strand breaks including USP3, USP28 and BRCC36. BRCC36 antagonizes RNF8-dependent ubiquitylation to maintain steady state levels required for appropriate signalling (Sobhian et al, 2007; Chen et al, 2006; Shao et al, 2009). USP3 is a chromatin-associated DUB that also antagonizes RNF8-mediated ubiquitylation (Nicassio et al, 2007). USP28 is a major regulator of DNA damage-induced apoptosis. It was shown that USP28 stabilizes CHK2, 53BP1 and a number of other DNA damage responsive proteins upon irradiation (Wu-Baer et al, 2003).

4.4 Crosstalk between post-translational modifications in response to DNA damage

Recent researches suggest that a crosstalk exists among multiple protein modifications, which collaboratively to regulate signal transduction of DNA damage and genetic stresses. p53, is subjected to multiple posttranslational modifications in response to genotoxic stress, which results in the accumulation of p53 and triggers its transcriptional activities. The damage-induced phosphorylation of p53 seems to be a signal for subsequent acetylation, because phosphorylation enhances its association with the CBP/p300 and PCAF to induce p53 acetylation in response to DNA damage, which results in p53 acetylation and further stabilized (Wang, 2008). Recent reports revealed that the Set8/Pr-Set7 methyltransferase suppresses p53 function in response to DNA damage (Shi et al. 2007), and lysine methylation of p53 by Set7/9 methyltransferase is important for its subsequent acetylation, which results in stabilization of the p53 protein (Ivanov et al., 2007). We also demonstrate that Set7/9 interacts with Sirt1 and induces a decrease in binding of Sirt1 to p53, and this relatively enhances p53 transactivity(Liu et al, 2011).

Apart from the above mentioned, H2AX, a variant form of H2A, is known to be acetylated by Tip60 acetyltransferase following DNA damage. Acetylated H2AX is required for its subsequent ubiquitylation via the ubiquitin-conjugating enzyme UBC13 (Ikura et al., 2007).

They suggested that acetylation-dependent ubiquitination by the Tip60-UBC13 complex leads to the release of H2AX from damaged chromatin, which enhanced histone dynamics and in turn stimulates a DNA damage response.

5. Conclusion

In summary, instability of genome is a constant problem of organisms. The coordination of DNA damage response (DDR) processes is required to maintain cellular viability and prevent diseases. The ATM and ATR protein kinases are master regulators of the DNA damage response. To further understand the molecular mechanisms through which the DDR operates, elucidate the genetic interactions between different DDR pathways and between DDR pathways and other cellular pathways, will be helpful for therapeutic strategies to treat many human disease.

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7. Abbreviations

The abbreviations used are: ATM, ataxia-telangiectasia mutated; ATR, ATM and Rad3-related; Chk, Checkpoint kinase; IR, ionizing radiation; PIKK, phosphoinositide-3 kinase-related kinases; ATR-IP, ATR-interacting protein; ROS, reactive oxygen species; DSB, double strand break ; 5-Aza-CdR,5′-aza-2′-deoxycytidine; DDR, DNA damage response; MRN, Mre11/Rad50/Nbs1; ssDNA, single strand DNA; 9-1-1, Rad9-Rad1-Hus1; FHA, forkhead-associated; BRCT, BRCA1 C-terminal; PRC1, Polycomb-repressive complex 1; DUBs, de-ubiquitylating enzymes.

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The study of DNA advanced human knowledge in a way comparable to the major theories in physics, surpassed only by discoveries such as fire or the number zero. However, it also created conceptual shortcuts, beliefs and misunderstandings that obscure the natural phenomena, hindering its better understanding. The deep conviction that no human knowledge is perfect, but only perfectible, should function as a fair safeguard against scientific dogmatism and enable open discussion. With this aim, this book will offer to its readers 30 chapters on current trends in the field of DNA replication. As several contributions in this book show, the study of DNA will continue for a while to be a leading front of scientific activities.

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