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Targeting DNA damage response: Threshold, chromatin landscape and beyond

Stefania Gonfloni *

Department of Biology, University of Rome "Tor Vergata", via della Ricerca Scientifica, I-00133 Rome, Italy

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ABSTRACT

Cells are continually exposed to DNA assaults from exogenous and endogenous sources. To maintain genomic integrity, cells have evolved a highly conserved mechanism for repairing DNA lesions and, in particular, DNA double strand breaks (DSBs). Emerging evidence indicates that DNA repair/signaling machinery acts in an integrated fashion with chromatin structure at damaged sites. This review focuses on the interplay between histone modifications and the chromatin-mediated response to DNA damage.

Contents

1. Introduction

Maintaining genomic integrity in response to DNA assaults is an essential process for living cells (Jackson & Bartek, 2009; Ciccia & Elledge, 2010; Negrini et al., 2010). To achieve this goal, cells use dynamic signaling networks that can sense, interpret and respond to various DNA stressors (Bekker-Jensen et al., 2010). Signal duration and amplitude of stresses evoke very complex patterns of protein–protein interactions. Canonical DNA damage kinases trigger several posttranslational modifications of DNA signaling proteins (Al-Hakim et al., 2010; Bensimon et al., 2011). These modifications show a dynamic behavior where signals of phosphorylation are interpreted through ubiquitin- (or Sumo-) mediated signal decoding (van Attikum & Gasser, 2005; Ramaekers & Wouters, 2011). The consequence is a timely recruitment (and disassembly) of large complexes near the damaged site (Panier & Durocher, 2009; van Attikum & Gasser, 2009; Ulrich & Walden, 2010). The first modification induced by DNA damage affects the histone variant H2AX on S139 to form γ-H2AX. S139-

Abbreviations: DSBs, double strand breaks; γ-H2AX, phosphorylation of histone variant H2AX in Ser139; SUMO, small ubiquitin modifier; ROS, reactive oxygen species; IR, ionizing radiation; Tip60, Tat-interactive 60; HAT, histone acetyltransferase; HDAC, histone deacetylase; NHEJ, non homologous end joining; HR, homologous recombination; DDR, DNA Damage response; NER, nucleotide excision repair; BER, base excision repair; MMR, mismatch repair; HP1, heteochromatin Protein1; INO80, inositol requiring 80; SWR1, sick with Rat8 ts; FHA, forkhead-associated; BRCT, breast cancer-terminal; UBD, ubiquitin binding domain; SIM, sumo interacting motif; MDC1, mediator of DNA damage checkpoint 1; BRCA1, breast cancer 1, early onset; FACT, facilitates chromatin transcription; SIRT1, sirtuin (silent mating type information regulation 2 homolog)1 deacetylase; WSTF, Williams–Beuren syndrome transcription factor tyrosine kinase; EYA, eyes absent protein phosphatase; MOF, males absent on the first; ATM, ataxia telangiectasia; MRN, Mre11/Nbs1/Rad50 complex; RNF8, RING finger protein 8; UBC13, ubiquitin-conjugating enzyme E2 13.

* Tel.: +39 06 72594319; fax: +39 06 2023500.
E-mail address: Stefania.Gonfloni@uniroma2.it.

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phosphorylation induces a massive accumulation of proteins in γ-H2AX foci (Stucki et al., 2005). The latter are microscopically visible aggregates, present in large segments of chromatin flanking the lesions, where the balance of opposing enzymes drives targeted recruitment, protein-protein interactions and posttranslational modifications (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Wang & Elledge, 2007; Doil et al., 2009; Stewart et al., 2009). Most of these enzymes are highly connected "hub" proteins interacting in complex regulatory circuits that allow temporary local clustering and reversibility of the interactions (Maiani et al., 2011). A second layer of complexity of DNA damage signaling relies on its profound impact on chromatin status (Gasch et al., 2001; Rieger & Chu, 2004; Reinhardt et al., 2011). At first glance, the DNA damage response induces a global repression of transcription (Vichi et al., 1997; Svejstrup, 2002) followed by a delayed transcriptional response, prolonging cell cycle arrest (Fei & El-Deiry, 2003; Elkon et al., 2005). In recent years, posttranscriptional control circuits are emerging as a third level of regulation of DDR signaling networks, recently reviewed by Boucas and co-workers (Boucas et al., 2012). They involve either RNA-binding proteins (RBPs) or non-coding RNAs, each of them impacting on protein biosynthesis (Fan et al., 2002; Matsuoka et al., 2007; Paulsen et al., 2009; Francia et al., 2012).

Chromatin structure and histone modifications are actively interconnected elements controlling the mechanisms underlying genome-integrity maintenance. While it appears intuitive that chromatin compaction protects DNA from lesions, chromatin state plays a central role for local signaling at the break points. For instance, "open" relaxed chromatin domains influence loading of DNA repair proteins onto chromatin near the site of the lesion (Xu & Price, 2011). Remodeling protein complexes (ATPase and Tip60 acetyl transferase HAT) can modulate chromatin accessibility and in combination with histone modifications promote subsequent chromatin ubiquitination (Lukas, 2010; Shanbhag et al., 2010). Thus, the dynamic landscape of chromatin, through a sophisticated combination of posttranslational modifications, may directly influence the choice of a specific DNA repair pathway adopted by the cells (Xu & Price, 2011; Chapman et al., 2012; Soria et al., 2012). In this review, I will focus specifically on the interplay between histone modifications, centered on H2AX phosphorylation, and the chromatin-mediated response to DNA damage.

2. When does DNA damage occur?

The major endogenous sources of DNA damage are reactive oxygen species (ROS) or unrepaired DNA lesions causing replication fork collapse in the cell (Ward & Chen, 2001; Zou & Elledge, 2003; Kryston et al., 2011; De Zio et al., 2012). DNA breaks also arise following treatment with exogenous genotoxic agents or ionizing radiation (IR) (Roos & Kaina, 2012). To counteract these different types of lesions cells explore multiple DNA repair pathways (Aziz et al., 2012). One type of lesion, the DNA Double strand break (DSB) is particularly dangerous for cells, as free DNA ends created by the lesion are susceptible to degradation or re-ligation, and promote genomic instability. DSBs can be generated by collapse of replication fork, ionizing radiation IR, exposure of specific compounds, and even during the processing of other lesions (Aziz et al., 2012). Under normal circumstances, DSBs are even programmed by the cell: in germ cells during meiotic repair (Keeney & Neale, 2006), or in lymphocytes during rearrangement of immunoglobulin genes (Dudley et al., 2005). DSBs can be repaired by two major pathways, homologous recombination (HR) and non homologous end-joining (NHEJ). In NHEJ, the broken ends are directly ligated in an error-prone manner (Lieber, 2010), frequently causing small insertions, deletions or substitutions at the break site. On the contrary, HR requires DNA end processing and a template (homologous DNA molecule) for error-free copying and repairing of the lesion.

3. How do cells respond to DNA insults?

Many studies done in the past on DDR have defined a hierarchical order among the different players, those, depending on their subcellular localization, are described as sensors, transducers, mediators and effectors (Bekker-Jensen et al., 2010). Sensors promptly respond to signals and are directly bound to chromatin, whereas transducers, mediators and effectors guide the DNA damage response events (Jackson & Bartek, 2009; Ciccia & Elledge, 2010; Negrini et al., 2010). Intuitively, a simple activation and regulation of this cascade occurs when DNA damage is not extensive and can be promptly repaired. When damage is prolonged, the balance of multi-layered connected pathways for DNA repairing and cell cycle arrest or initiating apoptosis (or senescence), leads cells to a decision point between survival or death (Ciccia & Elledge, 2010). DNA repair is essential for cell viability and normal growth, while irreparable damage definitely leads to a programmed cell death. To counter this, cells explore several different mechanisms for repairing DNA breaks. According to the type of damage, they use nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR). In addition, to repair the most dangerous lesions as DNA double strand breaks (DSB), cells can use two distinct pathways the (non-homologous end joining) NHEJ or HR (homologous recombination) repair systems (Aziz et al., 2012). Both compete one with each other and the choice between them is linked to the cell cycle phase and to the accumulation of specific markers. Many studies in the past have indicated that NHEJ pathway mainly occurs during G1-early S phase, even if it can be used during the whole cell cycle. On the contrary, HR repair is only used in late S-G2 phase (Takata et al., 1998; Shrivastav et al., 2008).

4. Stepwise response induced by DNA lesions

Emerging evidence suggests an active role of chromatin in DNA damage response (Bao, 2011; Luijsterburg & van Attikum, 2011; Lukas et al., 2011; Xu & Price, 2011; Miller & Jackson, 2012; Soria et al., 2012). Chromatin is a complex scaffold that compacts and organizes DNA in eukaryotic cells. Chromatin structure relies on a basic unit, the nucleosome. The nucleosome is formed by 146 base pair of DNA wrapped around a core composed by four different histones H2A, H2B, H3 and H4 (Campos & Reinberg, 2009). The central core of the nucleosome is formed by two H3-H4 dimers, surrounded by two H2A–H2B dimers (Campos & Reinberg, 2009). Chromatin structure is quite dynamic and can be modified through different mechanisms. Diverse classes of enzymes can modulate chromatin compaction. One class consists of large multi-protein complexes that need the energy of ATP hydrolysis to slide the nucleosomes or alter/exchange histone composition within the chromatin fibers. Another class of enzymes mediates covalent modifications of histone tails. Histone tails extend outward from the nucleosome and contain sites for regulatory modifications such as phosphorylation, ubiquitylation, methylation and acetylation. Histone modifications regulate chromatin functions. For instance, Lysine methylation (K4) of N-terminal tail of histone H3 (H3K4me) leads to gene activation and euchromatin formation (Margueron & Reinberg, 2010). On the contrary, methylation of histone H3 on K9 (H3K9me) creates an interaction motif for HP1 (Heterochromatin Protein 1) proteins, and promotes the formation of heterochromatin, leading to gene silencing (Bannister et al., 2001; Grewal & Jia, 2007). Changes in histone modification, implicated in the switch from euchromatin to heterochromatin, are regulated by pair-opposing enzymes that allow the dynamic rewriting of histone marks as histone acetyl transferases (HATs) and histone deacetylases (HDACs), HATs/HDACs, and (histone methyltransferase (HTMs) and histone demethylases (HDMs) HTMs/HDMs, also by DNA methyltransferase (Gallinari et al., 2007). Histone modifications, when present in specific combinations with other histone marks, can form a specific 'code' for recruiting some important effectors required for signaling

amplification and/or chromatin remodeling (Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004). Often, some histone marks compete one with each other or even for the same residue. This is particularly relevant for lysine residue, which can be alternatively modified by ubiquitylation, sumoylation, acetylation or methylation. Posttranslational modifications of histone by changing the charge of amino acid residue can alter the stability of the interaction with DNA or with other charged protein interfaces (Cairns, 2005; Kouzarides, 2007; Shahbazian & Grunstein, 2007; Campos & Reinberg, 2009; Suganuma & Workman, 2011).

In addition, histone modifications alter nucleosome composition, promoting the release of phospho-H2A histone from damaged chromatin (Ikura et al., 2007), through a histone variant exchange event. This event is regulated by an opposing action of two INO80 and SWR1 remodelers (Papamichos-Chronakis et al., 2006). Interestingly, enrichment of certain histone variants (e.g. H2AX) localizes sparse specific domains within chromatin fiber. Thus, differential expression of histone variants throughout the cell cycle and their respective positioning/localization onto chromatin (Talbert & Henikoff, 2010; Boyarchuk et al., 2011; Szenker et al., 2011) critically influence DNA damage response. In addition, histone chaperones (in synergy with ATP-dependent remodeling factors) (Clapier & Cairns, 2009) regulate the exchange with free unlabeled histones acting as "erasers" for specific posttranslational histone modifications. In a wide sense, histone variants (with their posttranslational labels) could be considered a "removable/temporary" platform for assembling local signaling circuitry onto chromatin. The amplitude and duration of such circuitry is likely regulated by feedback control and contribute to the fine-tuning of the DDR and to the maintenance of genome integrity.

5. Dynamics of histone modifications influences chromatin structure

Chromatin packaging is variable, typically distinguished in two states: a condensed (heterochromatin) and a more open (euchromatin) structure. Euchromatin is an accessible, gene rich and transcriptionally active region. Histones within euchromatin are highly acetylated and H3 is methylated on K4 and K36 (H3K4me and H3K36me2) (Barski et al., 2007; Guenther et al., 2007). On the contrary, heterochromatin has low gene density and instead contains many repetitive sequences (de Wit et al., 2007; Peng & Karpen, 2008). Histones within heterochromatin are poorly acetylated and H3 is highly methylated on K9 and K36 (H3K9me3; H3K36me3) (Pokholok et al., 2005; Vakoc et al., 2006; Guenther et al., 2007; Peng & Karpen, 2008). Posttranslational modifications of histones (PTM) can act as binding motif for proteins containing PTM-binding domain (Yun et al., 2011). For instance, bromodomains bind specifically to acetylated lysine residues, whereas chromodomains recognize methylated lysine residues (Kouzarides, 2007; Ruthenburg et al., 2007; Shahbazian & Grunstein, 2007). Other important PTM-binding domains present in DDR proteins include FHA or BRCT (breast cancer1, early-onset) domains (Mohammad & Yaffe, 2009) that recognize phosphorylated epitopes on target proteins as well as UBD (ubiquitin binding domain) domains and SIM (sumo interacting motif) motifs that bind ubiquitin and SUMO respectively (Kerscher et al., 2006; Hofmann, 2009).

The first evidence that chromatin is modified at site of DNA break came from the discovery that the histone variant H2AX is phosphorylated on its C-terminal tail following DNA damage (Miller & Jackson, 2012). Exposure of cells to DNA damaging agents activates phosphorylation of many target proteins (Bensimon et al., 2011). However, S139-phosphorylation of histone variant H2AX is considered one of the early markers of the DDR. This modification modulates the H2AX-interaction with DNA and promotes assembly of signaling complexes onto chromatin. While, there is a little evidence that γ-HAX has been involved in DNA repair *per se*, it plays a central role for the initiation and amplification of DNA damage signals. Signaling at DSBs induces γ-H2AX, eliciting timely engagement of MDC1 (mediator of DNA damage checkpoint 1), 53BP1 (p53 binding protein 1) and BRCA1 (breast cancer 1, early onset). Bidirectional spreading of γ-H2AX far from DNA lesions further enhances damage signaling and helps to delineate the chromatin region involved in DDR (Yuan et al., 2010). Silenced chromatin regions are not permissive for γ-H2AX spreading (Kim et al., 2007) and H2AX dynamics within the nucleosome are coupled to its various posttranslational modifications. Besides phosphorylation, other modifications such as ubiquitylation and acetylation of H2AX variant promote the recruitment of DDR proteins near the breaks. DNA damage-dependent modifications (i.e. poly ADP-rybosylation) of histone chaperone FACT (Facilitates Chromatin Transcription) inhibit its interaction with nucleosomes reducing the H2AX/H2A exchange (Du et al., 2006; Heo et al., 2008). On the contrary, the Tip60 complex (Tat-interactive protein 60), is a chromatin modifier and acetylates H2AX, enhancing its mobility within chromatin (Ikura et al., 2007). Interestingly Tip60-mediated acetylation (on K5) is required for the subsequent ubiquitination of H2AX (on K119) by RNF8/UBC13 (ubiquitin-conjugating enzymes) (see Fig. 1) (Ikura et al., 2007). In mammalian cells, SIRT1 (a protein deacetylase) negatively regulates Tip60-mediated acetylation of histone H2AX (Yamagata & Kitabayashi, 2009). Conversely, in yeast, a remodeling factor INO80 (Inositol requiring 80) retains phospho-H2A within the nucleosome (Papamichos-Chronakis et al., 2006). In combination with remodeling factors that promote γ-H2AX eviction from chromatin, several protein phosphatases can also negatively regulate the function of γ-H2AX by promoting its dephosphorylation (Nazarov et al., 2003; Chowdhury et al., 2005; Keogh et al., 2006; Chowdhury et al., 2008). The histone variant H2AX is also decorated by additional modifications which contribute to chromatin response to DNA damage (reviewed by Miller & Jackson, 2012). H2AX is phosphorylated on its C-terminal tyrosine residue by WSTF, a non canonical tyrosine kinase (Xiao et al., 2009). Following DNA damage, this phoshorylation is removed by the phosphatase EYA (Cook et al., 2009). The pair-opposing enzymes WSTF or EYA are both important for an effective DDR, pointing out the relevance of this tyrosine modification for H2AX function (Cook et al., 2009; Xiao et al., 2009). In sum, the fine-tuning of H2AX modifications and its dynamics mediated by the effect of specific action of pair enzymes offers a tunable switch for DNA damage signaling events.

6. Timing and threshold for DDR

Exposure of cells to genotoxic compounds activates the phosphatidylinositol-3-kinase-related kinase (PI3KK) family of kinases (ATM, ATR and DNA-PKcs). Although the PI3K related kinases are considered important players in DDR, an unrelated tyrosine kinase c-Abl has more recently also associated with the activation of key upstream event of DDR (Gonfloni, 2010a; Meltser et al., 2011; Wang et al., 2011; Maiani et al., 2012). In response to DNA damage, PI3K related kinases mediate the phosphorylation of H2AX on S139 (γ-H2AX). However, other numerous modifications (acetylation and methylation) occur on core histones in response to DNA damage. Two key modifications occur on H4, K16 acetylation and K20 methylation respectively. H4K16Ac modification is mediated by Tip60 and MOF (Murr et al., 2006; Li et al., 2010). While Tip60 mediates H2A and H4K16 acetylation at the site of break (Fig. 1), MOF seems to control global level of H4K16ac and does not localize at DSB (Sharma et al., 2010). Interestingly, Tip60 can also be activated by a histone mark (H3K9me3) associated with heterochromatin (Sun et al., 2009; Sun et al., 2010). Lack of either HAT enzymes (Tip60 and MOF) causes defective HR and NHEJ repair, suggesting that both are required for efficient DSB repair. However, the precise mechanism by which acetylation of H4K16 promotes DNA repair still remains elusive. Interestingly, a combination of histone marks such as H2b-Ub (ubiquitilated H2B) and H4K16ac induces decompaction of nucleosome (Shogren-Knaak & Peterson, 2006; Fierz et al., 2011). Thus,

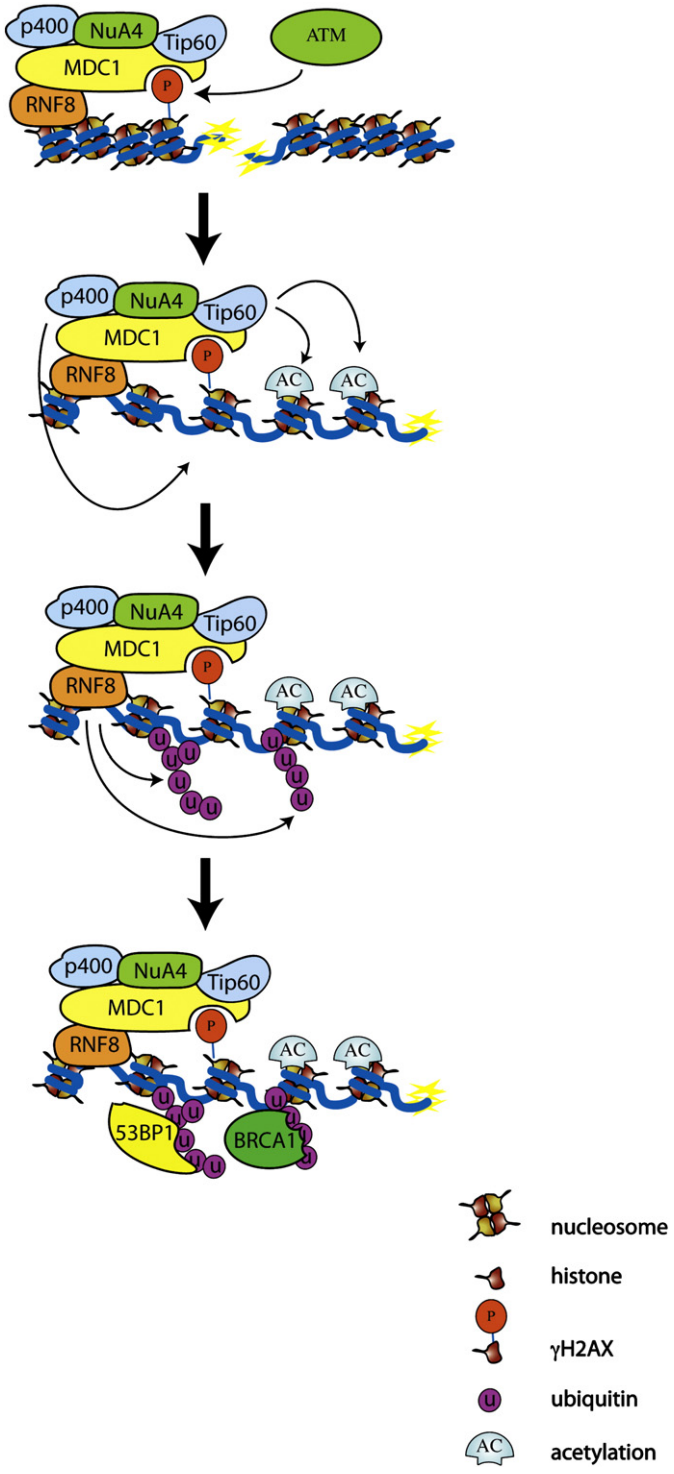

Fig. 1. Tip60-mediated acetylation of H2AX promotes Ubiquitin-dependent signaling at damage sites. H2AX phosphorylation by ATM provides a docking site for MCD1 and leads to the recruitment of ubiquitin ligase RNF8 and NuA4 complex at damaged sites. (NuA4 is a large complex form by Tip60, p400 motor ATPase and other subunits). Then, Tip60 mediates acetylation of histone H2AX, in combination with the action p400 ATPase, generates an open relaxed chromatin structure, facilitating ubiquitin-dependent signaling at the damage sites. Ac = acetylation, p = phosphorylation.

Tip60-mediated acetylation at DSB promotes instability of nucleosomes near the site of break. This could facilitate a shift of the local chromatin structure into an open relaxed conformation more permissive for the ubiquitin-dependent signaling at the damage site.

Posttranslational modifications of histones, both in the tails and in the core region affect the functional landscape of chromatin by regulating DNA accessibility. A key aspect of the role of histone modifications relies on their dynamic nature (Krebs, 2007); the precise timing of addition and removal of specific marks (or entire histone) determines a dynamic temporal regulation of chromatin functions (Krebs, 2007). H2AX-phosphorylation on S139 (γ-H2AX) is an early marker of DDR. Several studies indicate that γ-H2AX acts as beacon for proteins with dedicated phosphor-S/T binding domains (FHA, BRCT), promoting a sequential assembly of ubiquitin-dependent signaling cascades (see Fig. 1). Thus, DNA breaks initially promote repair and also a DNA signaling cascade for assisting repair (Yuan & Chen, 2010). At damaged site, the efficiency of signaling is enhanced by local concentration of factors. Signaling amplitude and duration are regulated through dynamic editing and removal of specific marks. This eventually could arrest the repair process for an alternative path leading to cell death. Most likely, survival of DNA-damaged cells strictly depends both on the removal of the lesion coupled with an efficient DNA damage signaling decay. This aspect is particularly crucial in the cellular context of perinatal oocytes more sensitive to genotoxic stress than somatic cells. (Gonfloni, 2010a; Gonfloni, 2010b; Maiani et al., 2012). In immature oocytes, pharmacological inhibition of c-Abl tyrosine kinase attenuates the toxic effect induced by chemotherapeutic drugs (Gonfloni et al., 2009; Maiani et al., 2012). Our studies indicate that c-Abl inhibition works on distinct levels of DNA damage signaling both at early time points reducing on γ-H2AX phosphorylation and then impinging on a downstream effector TAp63 (Gonfloni et al., 2009; Maiani et al., 2012). This supports the hypothesis that amplification of DNA damage signaling cascade leads germ cells towards death, as a default path, if not attenuated (Maiani et al., 2011).

7. Connections between DNA damage signaling and chromatin landscape

Now we move to the next point, how the H2AX dynamics, regulated by posttranslational modifications, in tandem with histone chaperones and remodelers contribute to the DDR? Recent evidence supports an active role of chromatin in DNA damage response. Chromatin compaction protects DNA from lesions. Heterochromatin, compared to euchromatin, is densely compact, transcriptionally silent and may act as barrier limiting access to all DDR factors (Soria et al., 2012). However, generation of DNA breaks and the early steps of DNA damage signaling and repair occur efficiently within the heterochromatin domains (Baldeyron et al., 2011; Chiolo et al., 2011; Jakob et al., 2011). Final steps of DNA signaling and repair (accumulation of RAD 51 and γ-H2AX spreading) are instead relocalized and confined in more accessible environment at the periphery of the heterochromatin region. The expansion of the heterochromatin facilitates the repositioning of damaged DNA near the surrounding euchromatin domains (Chiolo et al., 2011; Jakob et al., 2011; Baldeyron et al., 2011) to finalize late steps of DNA repair (Soria et al., 2012). Interestingly, a similar repositioning is observed during DNA replication (Quivy et al., 2004). It is possible that cells have evolved such mechanisms to prevent ectopic recombination between the repetitive sequences within heterochromatin, by restricting the processing of DNA ends at the periphery of heterochromatin (Quivy et al., 2004; Peng & Karpen, 2008; Chiolo et al., 2011). This could in turn prevent possible chromosomal rearrangements and genomic instability. How a sophisticated control on DNA accessibility is linked to DNA repair? ATM is a master regulator of DNA damage response. ATM mediates the phosphorylation of γ-H2AX promoting a signaling cascade, which leads to the assembly of DNA repair machinery and to activation of cell cycle checkpoints. However, how DNA breaks upregulate the activity of ATM is not completely clarified. Recent biochemical studies indicate that the autophosphorylation of ATM on S1981 is not the primary mechanism for ATM activation. This autophosphorylation is indeed dispensabile for

ATM function under some conditions (Lee & Paull, 2005; Dupre et al., 2006; Pellegrini et al., 2006). Most likely ATM activation is also mediated through binding with MRN (complex formed by mre11 nuclease, Rad50 ATPse and Nbs1) DNA binding complex (Uziel et al., 2003; Difilippantonio et al., 2005; Falck et al., 2005; Lee & Paull, 2005; Cerosaletti et al., 2006). Deletion of mre11, rad50 or nbs1 elements of MRN complex significantly reduces activation of ATM following DNA damage in vivo (Uziel et al., 2003; Difilippantonio et al., 2005; Falck et al., 2005; Cerosaletti et al., 2006). Recent studies indicate that Tip60 acetyltransferase is required for ATM activation (Sun et al., 2005). Tip60 and ATM form a complex in which Tip60 interacts with highly conserved FATC domain of ATM. This interaction in turn facilitates the acetylation of ATM on K3016 (Jiang et al., 2006; Sun et al., 2007). Tip60's chromodomain recognizes specifically H3K9me3 (Sun et al., 2009); and the binding with H3K9me3 increases Tip60 HAT activity through an allosteric mechanism. Mutations in the chromodomain binding motif prevent both the interaction between Tip60 and H3K9me3 and the upregulation of Tip60's HAT activity. The consequence is a reduction of acetylation and activation of ATM kinase activity mediated by Tip60 (Sun et al., 2009). Reduction of global H3K9me3 levels, by acting on opposing enzymes either on KDM4D demethylases (Whetstine et al., 2006) or Suv39 h1 and Suv39hu2 methyltransferase (Peters et al., 2001), significantly decreases Tip60 activation following DNA damage (Sun et al., 2009). In addition, cells with low level of H3K9me3 show an increased sensitivity to IR and genomic instability (Sun et al., 2009). Taken together, these observations suggest that the chromodomain functions as sensor for Tip60 activation, modulating both recruitment and HAT activity at DNA damage sites. Thus, a direct interaction between methylated histones and Tip60's chromodomain indicates that chromatin structure plays a role in DNA repair (Sun et al., 2010). Tip60 is stably associated with ATM in cells, and both proteins are recruited at DSBs (Fig. 2), most likely in an inactive state (Sun et al., 2005; Jiang et al., 2006). Recent evidence indicates that loss of functional MRN complex delayed the recruitment and activation of Tip60 after DNA damage (Sun et al., 2009). However the requirement of both MNR complex and Tip60 for ATM activation in vivo remains still elusive as well as the role of other potential effectors associated in the complex. Recent evidence indicates that Tip60 interacts with c-Abl tyrosine kinase both in vitro and in vivo and is an upstream c-Abl modifier in response to DNA damage. Interestingly, c-Abl acetylation mediated by Tip60 required an ATM-mediated phosphorylation of c-Abl on S465 (Jiang et al., 2011).

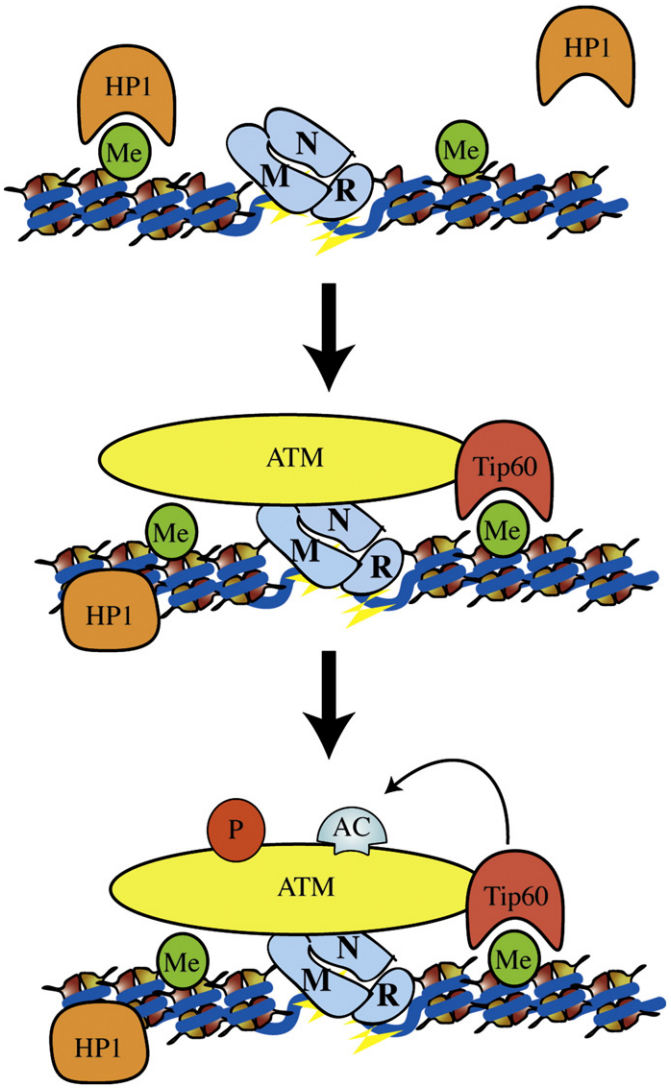

Fig. 2. Early steps of DNA damage response: a potential model for ATM activation by Tip60. Following DNA damage, MRN is recruited to DSB. In parallel, HP1 proteins are released from H3K9me3 (heterochromatin histone mark). MRN promotes targeted recruitment of the inactive ATM-Tip60 complex at DSBs. This event facilitates the interaction between the chromodomain of Tip60 and H3K9me3, enhancing Tip60's HAT activity through an allosteric mechanism. Interaction between MRN and Abl, together with acetylation of ATM mediated by Tip60, activates the kinase activity of ATM. Me = methylation, Ac = acetylation, p = phosphorylation.

8. Concluding remarks

In conclusion, chromatin is emerging as an integral player in the DDR (Soria et al., 2012). Cells have evolved dedicated signaling and repair machinery to control the chromatin structure facilitating (or preventing) DNA access in a dynamic way at the site of damage and nearby. This sophisticated machinery includes enzymes involved in posttranslational modifications of histones, incorporation of histone variants and ATP-dependent chromatin remodeling. Emerging evidence indicates that all these three classes of components are direct players in DNA damage response induced by DSBs acting in an integrated fashion. Histone deacetylases (HDACs) promote chromatin condensation and are considered promising targets for cancer therapy because their inhibition is preferentially toxic for some cancer cells (Johnson et al., 2002; Minucci & Pelicci, 2006). More sophisticated techniques based on ChIP assay and the development of powerful DSD-inducing systems are rapidly improving our understanding of DSB repair processes (Polo & Jackson, 2011). Undoubtedly, this will provide new hints for the development of targeted therapies for DDR in a global integrated fashion.

Conflict of interest

The author declares no conflict of interest.

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