Published in final edited form as:

DNA Repair (Amst). 2015 December ; 36: 8–12. doi:10.1016/j.dnarep.2015.09.002.

Chromatin perturbations during the DNA damage response in higher eukaryotes

Christopher J. Bakkenist^a and Michael B. Kastan^{b,*}

^aDepartments of Radiation Oncology and Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Hillman Cancer Center, Research Pavilion, Suite 2.6, 5117 Centre Avenue, Pittsburgh, PA 15213-1863, USA

^bExecutive Director, Duke Cancer Institute, William W. Shingleton Professor of Pharmacology and Cancer Biology, Duke University School of Medicine, 422 Seeley Mudd Building, Box 3917, Durham, NC 27 710, USA

Abstract

The DNA damage response is a widely used term that encompasses all signaling initiated at DNA lesions and damaged replication forks as it extends to orchestrate DNA repair, cell cycle checkpoints, cell death and senescence. ATM, an apical DNA damage signaling kinase, is virtually instantaneously activated following the introduction of DNA double-strand breaks (DSBs). The MRE11-RAD50-NBS1 (MRN) complex, which has a catalytic role in DNA repair, and the KAT5 (Tip60) acetyltransferase are required for maximal ATM kinase activation in cells exposed to low doses of ionizing radiation. The sensing of DNA lesions occurs within a highly complex and heterogeneous chromatin environment. Chromatin decondensation and histone eviction at DSBs may be permissive for KAT5 binding to H3K9me3 and H3K36me3, ATM kinase acetylation and activation. Furthermore, chromatin perturbation may be a prerequisite for most DNA repair. Nucleosome disassembly during DNA repair was first reported in the 1970s by Smerdon and colleagues when nucleosome rearrangement was noted during the process of nucleotide excision repair of UV-induced DNA damage in human cells. Recently, the multi-functional protein nucleolin was identified as the relevant histone chaperone required for partial nucleosome disruption at DSBs, the recruitment of repair enzymes and for DNA repair. Notably, ATM kinase is activated by chromatin perturbations induced by a variety of treatments that do not directly cause DSBs, including treatment with histone deacetylase inhibitors. Central to the mechanisms that activate ATR, the second apical DNA damage signaling kinase, outside of a stalled and collapsed replication fork in S-phase, is chromatin decondensation and histone eviction associated with DNA end resection at DSBs. Thus, a stress that is common to both ATM and ATR kinase activation is chromatin perturbations, and we argue that chromatin perturbations are both sufficient and required for induction of the DNA damage response.

*Corresponding author. Fax: +1 919 681 7385. bakkenistcj@upmc.edu (C.J. Bakkenist), michael.kastan@duke.edu (M.B. Kastan).

Conflict of interest

None

Keywords

ATM; ATR; Chromatin perturbations; Double-strand breaks (DSBs); Ionizing radiation (IR); Nucleolin

1. Introduction

There are a myriad of responses to DNA lesions that function to maintain genome stability [1]. The DNA damage response is a widely used term that encompasses all signaling initiated at DNA lesions and damaged replication forks as it extends to orchestrate DNA repair, cell cycle checkpoints, cell death and senescence. As such, the first description of a DNA damage signaling pathway response was the demonstration that a DNA damage-induced, p53-dependent cell cycle checkpoint was deficient in cells derived from individuals with ataxia telangiectasia and by the presence of subsequent specific, p53-dependent gene activation [2]. Additional mechanistic paradigms within this signaling pathway were rapidly identified as ataxia telangiectasia-mutated (ATM), the gene mutated in ataxia telangiectasia, was found to encode a protein kinase, and p21, a transcriptional target of p53, was determined to be an inhibitor of G₁-phase cyclin-dependent kinases (CDKs) [3–5]. It is now clear that ATM kinase activation results in p53 induction [6–8], p53 transactivation activity results in p21 transcription, and p21 causes G₁-phase CDK inhibition which delays cell cycle progression, similar to a series of events noted in *S. cerevisiae* where a G₂-phase arrest is dependent on the Rad9 gene [9]. Building on these concepts, pharmacologic inhibition of CDK4/6 using the selective kinase inhibitor PD0332991 causes a reversible G₁-phase arrest that has been associated with radiation protection in human fibroblasts, cancer cells, and mice [10,11]. Since these inception, a large number of mechanisms that contribute to the initiation and amplification of DNA damage signaling through the apical kinases ATM and ataxia telangiectasia and Rad3-related (ATR), together with the E3 ubiquitin ligases RNF8 and RNF168, have been appropriately woven into the DNA damage response (comprehensively reviewed in [12–14]). While ATM kinase remains a central player in DNA damage responses, it is also now clear that ATM kinase activity functions in other physiological processes as well, including insulin signaling and regulation of mitochondrial function [15–17].

2. Chromatin decondensation at DSBs and ATM kinase activation

Cell cycle checkpoint defects were initially described in cells derived from ataxia telangiectasia patients exposed to ionizing radiation (IR) [2,18]. ATM encodes a serine/threonine kinase that is a key regulator of DNA double-strand break (DSB) signalling and repair [5,19]. The generation of antibodies that recognize ATM only when it is in its autophosphorylated (serine-1981) activated state, allowed ATM kinase activation to be detected in primary human fibroblasts that were exposed to IR doses as low as 5 cGy [19,20]. Similarly, increased ATM kinase activation was observed in peripheral blood mononuclear cells of patients receiving stereo-tactic body radiation therapy, which were estimated to be exposed to 6 cGy IR, as they circulated through the irradiation field [21]. Sensing of DNA lesions occurs within a highly complex and heterogeneous chromatin

environment [22]. The ATM kinase is virtually instantaneously activated following introduction of DSBs and the MRE11-RAD50-NBS1 (MRN) complex, which has a catalytic role in DNA repair [23–25], and the KAT5 (Tip60) acetyltransferase [26–28] are required for maximal ATM kinase activation in cells exposed to low doses of IR. In cells the MRN complex binds directly to both DSBs and ATM and, at least *in vitro*, MRN is an allosteric activator of ATM kinase [29,30]. On the other hand, KAT5 is recruited to DSBs in a complex with ATM [27,28]. KAT5 binds to H3K9me3 and H3K36me3, which function as allosteric activators inducing acetylation of ATM on lysine-3016 [27,28], histone H2A and histone H4 [31,32]. Moreover, chromatin decondensation and histone eviction at DSBs [33–36] may be permissive for KAT5 binding to H3K9me3 and H3K36me3, followed by ATM kinase acetylation, activation and ATM kinase-dependent cell cycle checkpoints [37]. Consistent with the premise that chromatin perturbations in DNA adjacent to a DSB contribute to ATM activation, maximal ATM kinase activation in *Xenopus* egg extracts requires DNA regions of hundreds of base pairs flanking DSB ends [38]. Mutation of the ATM acetylation site (lysine-3016) blocks DNA damage-induced ATM kinase serine-1981 phosphorylation, ATM kinase signalling and ATM kinase-dependent cell cycle checkpoints [28]. Thus, KAT5 binding to H3K9me3, which is associated with inactive heterochromatin [39,40], and H3K36me3, which is associated with active euchromatin [41–43], and chromatin decondensation in DNA regions flanking DSBs are central to the molecular mechanisms that activate ATM kinase.

3. ATM kinase activation causes chromatin perturbations

The mobility of DSBs in the nucleus is low during the initial stages of DNA repair, and DNA damage signaling proteins (including MRE11) localize to sites of damage within minutes [44]. The MRN complex binds directly to both DSBs and ATM [29,30], and ATM phosphorylates histone H2AX on serine 139, generating γ H2AX in up to 1 megabase (Mb) of the chromatin fiber on either side of the DSB [45]. H2AX phosphorylation along the chromatin fiber requires the mammalian SWI/SNF chromatin remodeling complex [46]. Thereafter, the MDC1 scaffold protein binds directly to γ H2AX creating a platform that localizes MRN, ATM, and additional proteins including the adapter protein 53BP1 and the E3 ubiquitin-protein ligase RNF8 on the chromatin fiber on either side of the DSB [47–50]. 53BP1 binds H3K79me and chromatin decondensation has been implicated directly in the recruitment of 53BP1 to chromatin fiber on either side of the DSB [51]. Thus, ATM kinase activation is central to the modifications and, or, localization of potentially hundreds of protein molecules in ‘DNA damage signaling structures’ that cover approximately 1 Mb on the chromatin fiber on either side of the DSB, forming microscopically detectable DNA damage- or IR-induced foci. Different proteins have different rates of dynamic exchange within IR-induced foci indicating that the ‘DNA damage signaling structures’ are highly ordered [47,52] and this exchange appears to be regulated, at least in part, by ATM kinase activity [53,54]. Thus, ATM kinase signaling is maintained in highly ordered chromatin perturbations that are coincident with γ H2AX.

The bromodomain protein Brd4 isoform B is a chromatin factor that restricts the size, but neither the incidence nor resolution of γ H2AX, 53BP1 and phosphorylated ATM in the DNA damage signaling structures described above in cells exposed to 2 Gy IR [55]. Brd4

isoform B binds acetylated histones and chromatin-binding proteins including SMC2 (structural maintenance of chromosomes protein 2) and SMC4 [55]. Brd4 isoform B localizes SMC2 and SMC4 to exposed acetylated histones causing chromatin condensation, which limits ATM kinase signaling [55]. Thus, while chromatin is hypersensitive to nuclease digestion following IR, indicating increased accessibility of the nuclease to the linker DNA between the nucleosomes [56,57], dynamic chromatin remodeling, decondensation and condensation, determine the size of DNA damage signaling structures and ATM signaling on the chromatin fiber on either side of the DSB. Heterochromatin is relatively refractory to H2AX phosphorylation [58] and the resolution of DNA damage signaling structures in heterochromatic regions is slower than that in euchromatic regions [59]. ATM kinase phosphorylates the transcriptional corepressor Kruppel-associated box (KRAB)-associated protein (KAP)-1 causing chromatin decondensation at DSBs in heterochromatin [33]. Moreover, ATM kinase is essential for the resolution of DNA damage signaling structures in heterochromatin that persist at late time points (24 h) after insult [59].

Changes in chromatin structure may differ across multiple regions in the chromatin fiber around the DSB. For example, the ATM kinase signaling at DSBs that represses proximal transcription in *cis* over several kbp of the DNA fiber is associated with transcriptional silencing through the ubiquitination of histone H2AK119 by RNF8 and RNF168 [60]. ATM kinase activity also causes RNA polymerase II to stall and this is associated with an inhibition of dependent chromatin decondensation at regions distal to DSBs [60]. This long-range inhibition of chromatin decondensation is only associated with active transcription and may be related to a role for ATM in DNA repair. ATM kinase activity is also required for the mobilization and pairing of homologous chromosomes in G₁-phase cells at the site of a DSB, a phenomena referred to as “chromosome kissing” [61,62]. Contact between homologous chromosomes in G₁-phase cells is only observed when a DSB is induced in a sequence associated with active transcription [62]. Thus, ATM kinase activity induces local and long range chromatin perturbations.

4. Chromatin perturbation and ATM kinase activation

The exquisite sensitivity and kinetics of ATM kinase serine-1981 phosphorylation after IR led to the hypothesis that ATM kinase may be activated by chromatin perturbations [19]. Exposure of cells to a number of non-DNA-damaging agents such as hypotonic culture media, chloroquine and histone deacetylase inhibitors causes chromatin perturbations without inducing DSBs. During these treatments ATM kinase activation could be detected in the absence of detectable H2AX phosphorylation [19], which was the most sensitive assay for detecting DSBs at that time. Since this initial observation, a number of laboratories have further advanced the paradigm that the DNA damage response can be induced by chromatin perturbations in the absence of DNA damage. Treating cells with histone deacetylase inhibitor trichostatin A (TSA), in the absence of exogenous sources of DNA damage is sufficient to induce ATM acetylation, ATM kinase serine-1981 phosphorylation and ATM kinase-dependent cell cycle checkpoints [19,37]. Moreover, activation of ATM in cells treated with chloroquine or other chromatin modifiers does not require the MRN complex [25,37]. Consequently, immunostaining for phosphorylated ATM protein is diffuse rather than showing the typical accumulation of foci after induction of DNA strand breaks [19,25].

However, pre-treating cells with TSA potentiates IR-induced ATM kinase serine-1981 phosphorylation [19,37].

While chromatin decondensation may be permissive for KAT5 binding to H3K9me3 and H3K36me3, the c-Abl-dependent KAT5 tyrosine-44 phosphorylation is an additional mechanism of chromatin sensing associated with ATM kinase activation [37]. KAT5 tyrosine-44 phosphorylation is induced and then stabilized as phosphorylated KAT5 accumulates in chromatin of cells in which H3K9me3 is exposed [37]. As such, chromatin perturbations, which are yet to be defined beyond exposed H3K9me3, can induce ATM kinase activation and ATM kinase-dependent cell cycle checkpoints in the absence of detectable DNA damage.

Interestingly, NBS1, MRE11, MDC1 or the large fragment of ATM (amino acids 1300–3060), fused to lacR and localized to 256 repeats lacO in chromatin is sufficient to induce ATM kinase-dependent H2AX phosphorylation indicating that the functional structure can be assembled on MDC1 in the absence of a DSB [63]. Hence, the concentration of these proteins in a single location is sufficient to induce ATM serine-1981 phosphorylation, ATM kinase signaling and an ATM kinase-dependent G2/M-phase cell cycle checkpoint in the absence of an exogenous source of DNA damage [63]. The concentration of DNA damage signaling proteins on the lacO operator is unlikely to coincide with an endogenous DSB [63]. Thus, chromatin perturbations are sufficient to induce ATM kinase activation and ATM kinase-dependent cell cycle checkpoints.

5. DNA repair in the context of chromatin

Nucleosome disassembly during DNA repair was first reported in the 1970s when nucleosome rearrangement was noted during the process of nucleotide excision repair of UV-induced DNA damage in human cells [64,65]. Subsequently, similar conclusions were extended to studies using yeast [66,67]. More recently, to follow repair of DSBs in chromatin, investigators created DSBs at defined sites in the human genome using the homing endonuclease I-PpoI [36]. It was demonstrated that partial disruption of nucleosomes (loss of histones H2A and H2B) occurred at sites of DNA breakage during repair by non-homologous end-joining [36]. The authors identified the multi-functional protein ‘nucleolin’ as the relevant histone chaperone that was required for the removal of H2A and H2B [36]. Furthermore, they showed that nucleosome disruption was required for recruitment of repair enzymes to the DSB and for DNA repair [36]. More specifically, nucleolin was targeted to the DSB by binding to Rad50 protein of the MRN complex, accompanying MRN to the sites of DNA breakage [36]. Thus, chromatin perturbations are involved in both, ATM kinase activation (see above) and DNA repair [68].

6. ATM kinase activation and metabolic signaling

In addition to agents that modulate chromatin (see above) ATM can also be activated in some cell types by insulin treatment [15,16], mitochondrial electron transport chain inhibition [17] or by other metabolic stresses (Brown, Scarbrough and Kastan, unpublished). Whether there is a single common mechanism for ATM activation following the induction of DSBs, chromatin perturbations, and metabolic stresses, remains to be clarified.

7. Ataxia telangiectasia and Rad3-related (ATR)

The ATM-related protein kinase, ATR, is activated at single-stranded DNA (ssDNA) and at junctions between ssDNA and double-stranded DNA (dsDNA) that are associated with DSBs and many DNA lesions that interfere with DNA replication [12–14]. When ssDNA is generated, it is rapidly coated with replication factor A (RPA), which is a ssDNA-binding protein complex. The ATR-interacting protein, a regulatory partner of ATR, binds directly to RPA-coated ssDNA [69] and the RAD17-RFC2-5 complex recruits the RAD9-RAD1-HUS1 (9-1-1) complex to dsDNA-ssDNA junctions [70]. TOPBP1, an allosteric activator of ATR–ATRIP, is then recruited [71,72]. TOPBP1 phosphorylation by ATM and ATR on serine-1131 enhances the interaction between TOPBP1 and ATR–ATRIP [73]. Overexpression of a small domain of TOPBP1 induces ATR kinase activation, ATR kinase-dependent cell cycle checkpoints, and senescence in the absence of exogenous DNA damage [74]. While ATR kinase activation was initially only associated with stalled and collapsed replication forks [75], it was later shown that ATR kinase is also activated at DSBs and that maximal ATR kinase activation at DSBs requires DNA end resection [76,77]. Thus, central to the mechanisms that activate ATR, outside of a stalled and collapsed replication fork in S-phase, is chromatin decondensation and histone eviction prior to DNA end resection [77], and a stress that is common to both ATM and ATR kinase activation is chromatin perturbations.

8. Concluding remarks

ATM kinase signaling is defined by chromatin perturbations (Fig. 1). Activation of ATM kinase requires the assembly of ‘DNA damage signaling structures’ on chromatin, and this is associated with chromatin decondensation and remodeling. But ATM kinase signaling also induces chromatin perturbations, which differ across multiple chromatin regions. In euchromatin, ATM kinase signaling is associated with transcriptional silencing and inhibition of RNA polymerase II transcription-associated chromatin decondensation and chromosome kissing at the site of a DSB in G₁-phase cells. In heterochromatin, ATM kinase signaling is associated with chromatin decondensation and the resolution of DNA damage signaling structures. Surprisingly, chromatin perturbations are both sufficient and required for activation of the DNA damage response.

Once activated, ATM and ATR phosphorylate virtually identical consensus target sequences [78], and hundreds of potential substrates have been identified in human cells [79–81]. While gene ontology analysis identified DNA replication, recombination and repair, and chromatin remodeling substrates as the most common [54,79], substrates that impact every aspect of cell physiology have been identified and the significance of many phosphorylations remains an open question. Nevertheless, a recurrent theme in the DNA damage response is chromatin perturbations, as first innovated and elucidated by Smerdon and colleagues over the years.

Acknowledgments

This review is not intended to be a comprehensive review of mechanisms encompassed by the DNA damage response. We apologize to friends and colleagues whose work we have not cited due to space restrictions. Work in the authors laboratories is supported by grants CA148644 (CJB) and CA157216, CA159826 and ES005777 (MBK).

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Fig. 1. ATM is activated by chromatin perturbations

A. Chromatin decondensation and histone eviction at DSBs may be permissive for KAT5 binding to H3K9me3 (Me) and increased KAT5 acetyltransferase activity. KAT5 acetylates ATM (dimer) on lysine-3016 (A) inducing ATM kinase activity, autophosphorylation on serine-1981 (P) and dissociation of the dimer. Maximal ATM activation at DSBs requires KAT5 and the MRN complex. In addition, KAT5 acetylates histones and ATM phosphorylates histones together with several other substrates related to DNA replication, recombination, repair and chromatin remodeling, including KAP-1. MDC1 is recruited to H2AX phosphorylated on serine-139 (P) and serves as a platform for 53BP1, ATM and RNF8 which ubiquitinates histones (U).

B. Nucleolin, a histone chaperone, is recruited to DSBs with the MRN complex. KAP-1 promotes chromatin decondensation.