RESEARCH HIGHLIGHT

Histone code in the cross-talk during DNA damage signaling

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Cells and organisms are targets of endogenous and exogenous genotoxic agents. Those hits induce different lesions of the DNA including single and double strand breaks, which in turn trigger the appropriate response. Deficiencies in DNA damage response lead to deregulation of critical cellular processes such as proliferation, cell cycle arrest, and apoptosis, and may favor induction of mutations and genomic instability thus facilitating tumor development. Double strand breaks (DSBs) are thought to be the most dangerous lesions for genomic integrity as they facilitate chromosome translocations. These lesions occur in a specific context where the DNA is wrapped around histones to constitute the chromatin. The structure and function of chromatin are modulated by covalent attachment of acetyl and methyl groups (histone marks) to core histones, specialized proteins that form building blocks of chromatin (nucleosomes). Traditionally chromatin can be distinguished by heterochromatic regions which are protein dense where histones are methylated and with repressive marks and euchromatic regions which are more active and mainly associated with acetylation of histones and specific methyl marks considered as active marks.

Recent studies have shown that the structure of chromatin and chromatin remodelers are playing a preponderant role in the repair of DSB [1]. These studies have revealed that recruitment of repair protein complexes may be mediated by chromatin modifiers involved in acetylation and deposition of histone marks [2, 3]. The molecular events and dynamics of the recruitment of DNA repair proteins at the site of the break are yet to be defined. Tip60, a histone acetyltransferase, mediates the acetylation of histones that was found to be important during the DNA repair process. Tip60 was also shown to be recruited at the site of the break and to acetylate histones which promotes the relaxation of the chromatin and facilitates the access of repair proteins [2]. Interestingly, the proteins involved in the acetylation of histones were also found associated with the MRN complex (Mre11-Rad50-Nbs1) which is known to be the key component of the repair machinery that is recruited at DSB sites at early stages, however precise order of events remains poorly understood [3]. Recent work by the Brendan Price's laboratory indicated that Tip60 may play an important role in the activation of DNA damage response through acetylation of ataxia telangiectasia autated (ATM) protein kinase [4]. However, what promotes Tip60-mediated acetylation of histones and activation of ATM after DNA damage remained unclear.

In the present study Sun and col-

leagues [5] investigated how histones adjacent to DSB may regulate acetyltransferases activity of Tip60. Sequence alignment of Tip60 revealed the presence of chromodomain which may facilitate either the recruitment or the activation of Tip60 upon DNA damage. The authors used bleomycin as damaging agent to induce DSBs and investigated the acetylation activity of different Tip60 mutants after DNA damage. *In vitro* experiments revealed that the chromodomain of Tip60 is necessary for acetylation of histones as well as the activation of ATM signaling pathway. Previous work by the same group showed the existence of Tip60-ATM complex [4, 6], and since ATM is activated by MRN [7], the authors investigated the role of MRN in Tip60 activation. They observed that the silencing of the Rad50 subunit of MRN complex leads to a reduction of Tip60 activity and impaired recruitment of Tip60 at the site of the break. Thus, MRN as well as Tip60 chromodomain are both required for the acetyltransferase activity of Tip60, however the lack of chromodomain does not impair the loading of Tip60 to DSB, consistent with a role of the chromodomain for the activity and not for the recruitment of Tip60. The author hypothesized that the binding of the chromodomain to specific lysine residue may trigger the activation of Tip60. Chromodomains are methyl-lysine binding modules that are mainly associated with methyl-lysine residues

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of histone H3. Remarkably, the addition of methylated histone H3, more specifically tri-methylated lysine-9 of histone H3 (H3K9me3) and tri-methylated lysine-36 of histone H3 (H3K36me3), to Tip60 was able to activate the histone acetyltransferase activity while it was not the case for other methyl lysine residues. Furthermore, H3K9me3 showed a strong binding affinity for Tip60 whereas H3K36me3 exhibited a weaker affinity for Tip60 chromodomain, suggesting that H3K9me3 is the primary binding site for Tip60 chromodomain.

The role of H3K9me3 in DNA damage response was further addressed by *in vivo* experiments where impairment of the trimethylation of H3K9 was achieved by knockout of Suv39h histone methyltransferase or by overexpression of histone demethylases targeting H3K9me3. Even if the role of H3K36me3 remains unclear, the results confirmed *in vitro* findings on the requirement of H3K9me3 for acetyltransferase activity of Tip60 but not for its recruitment. Overexpression of H3K9me3 histone demethylase resulted in impaired histone methylation and attenuated activation of the DNA damage signaling pathway after DNA damage. To better understand the interaction and induction of Tip60 activity after DNA damage, the authors performed immunoprecipitation experiments which showed that Tip60 is bound to H3K9me3 upon induction of DNA damage. H3K9me3 is associated with the chromodomain of HP1 and casein kinase 2 (CK2) that mediates the removal of HP1 protein. Using chromatin fractionation and inhibitor against CK2, the authors further showed that release of HP1 β is required for a proper activation of the ATM DNA damage signaling pathway. These findings provide an important insight into the early events of DNA damage response and DNA repair triggered by histone modifications.

Together, the results obtained by Sun and colleagues showed that specific histone modifications (H3K9me3 and

to some extent H3K36me3) trigger the activation of Tip60 acetyltransferase highlighting an interaction (cross-talk) between histone marks and histone modifying enzymes during DNA damage response. Since other previous studies showed that histone marks may serve as docking sites for the binding of early repair protein (such as 53BP1 to the methylation of a specific lysine residue K79 of histone H3 [8]), the present study provides evidence for an independent mechanism involving histone marks in the early stage of DNA damage activation. However, this study also raises several other important questions regarding the role of histone marks and their interdependence during the activation of ATM-dependent signaling cascade and DNA damage response including DNA repair. Histone methylation of H3K9 is traditionally associated with transcriptionally silenced chromatin, therefore the finding that this mark serves as docking site for and activation of Tip60 during DNA damage response suggests that specific histone marks may play a major role in the coordination of gene transcription and DNA repair, two distinct and often conflicting processes. Further studies are needed to investigate the timing and exact order of events (including the recruitment of histone modifying molecules/complexes and histone modifications) during early changes of DNA damage response and DNA repair. It is unclear whether Tip60 functions in the context of ATM activation as a free molecule or as part of multisubunit HAT complex. It also remains to be determined whether the type of DNA damage (single strand vs double strand breaks) and DNA repair pathway (homologous recombination vs non-homologous end joining) trigger different histone modifications and/or combination thereof. The study by Sun *et al.* suggests an intriguing possibility that Tip60 may be preferentially activated by DNA damage occurring in the context of heterochromatin. Although this is consistent with previous stud-

ies showing that different chromatin compaction states may have differential requirements for ATM activity [9, 10], further studies are needed to test if Tip60 activity following DNA damage is restricted to heterochromatic regions. It will also be interesting to examine whether Tip60 activity triggered by DNA damage cross-influences its transcriptional role. This work thus opens important perspectives for novel studies on the mechanisms of DNA damage response and tumor development, and these unresolved issues warrant further studies.

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