Tip60-ing the balance in DSB repair

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The tumour suppressor Tip60 is a histone acetyltransferase implicated in transcriptional control and DNA double-strand break repair. Tip60 binds to the heterochromatic histone mark H3K9me3, triggering acetylation and activation of DNA double-strand break repair factors.

Endogenous (metabolic) and exogenous (environmental) agents constantly induce DNA double-strand breaks (DSBs). Inefficient or inaccurate DSB repair results in the loss of genome integrity, leading to cellular transformation or apoptosis. An elaborate system has evolved that integrates DNA damage detection and cell-cycle checkpoint mechanisms to coordinate repair and cell division. Over the last few years, many different components of the homologous recombination (HR) and non-homologous end joining (NHEJ) DSB repair pathways have been defined. Also, numerous post-translational modification (PTM) signalling events in DSB repair have been uncovered[1]. Nevertheless, in many cases the exact mechanistic connection between different factors and the ordered events of DSB repair are still obscure. On page 1376 of this issue, Sun *et al.* provide evidence that a key component of the DSB repair pathway, ATM (ataxia-telangiectasia mutated) kinase, is activated after the histone acetyltransferase Tip60 is stimulated through interaction with a heterochromatic histone modification mark[2].

The eukaryotic genome is organized into repeating units of chromatin, which is composed of nucleosomes comprising complexes of the basic histone proteins H2A, H2B, H3 and H4. The addition of other factors, such as linker histone H1 and non-histone chromatin proteins, results in higher order chromatin organization and compaction, which can present a barrier to DNA replication, transcription and DSB repair. However, PTM of histones and ATP-dependent chromatin remodelling have evolved to reorganize stretches of chromatin, thereby allowing processing machinery — including DSB repair complexes — access to chromosomal DNA. Besides opening up chromatin for repair, it turns out that histone PTMs are essential for factor recruitment, signal propagation and, as the new work shows, the activation of enzymatic function.

A key question is how changes in chromatin structure are initiated at DSB sites (Fig. 1). In mammalian chromatin, about 10% of all nucleosomes contain the histone H2A variant H2AX instead of canonical H2A. Earlier work has shown that, following the induction of DSBs, the MRN complex (MRE11–RAD50–NBS1) binds to broken DNA ends and recruits ATM, ATR and/or DNA protein kinase (the PIK-family protein kinases), resulting in the initial phosphorylation of H2AX[1,3]. The adaptor protein MDC1 then associates with phosphorylated H2AX (γ-H2AX) and recruits additional activated ATM to the sites of DSBs[4,5]. This positive feedback loop leads to the expansion of the γ-H2AX region surrounding DSBs, to up to 2 Mb, and provides docking sites for other DNA damage and repair proteins, including 53BP1 and BRCA1. γ-H2AX incorporation is followed by hyperacetylation of H3 and H4, which seems to contribute to the opening and relaxation of chromatin. Downstream chromatin events include additional PTMs of histones, such as ubiquitylation and sumoylation of γ-H2AX, H2A and H2B. These histone PTMs are thought to have roles in histone exchange, as well as recruitment of the chromatin remodelling complexes INO80 and SWR1 (ref. 1).

As PIK-family kinases are early inducers of DSB chromatin changes and repair pathways, the exact mechanisms that result in their activation are of great interest. Biochemical studies have shown that inactive ATM protein dimers dissociate into active kinase monomers after autophosphorylation[6,7]. This autophosphorylation is stimulated by acetylation of ATM by the histone acetyltransferase (HAT) and tumour suppressor protein Tip60 (ref. 8). Tip60 and ATM form a stable complex that is recruited to DSBs. Although it was known that Tip60 activity is stimulated upon DNA damage, the exact mechanisms of enzyme activation were unknown. The work by Sun *et al.* now provides the missing link in the activation pathway. Binding of Tip60 through a chromodomain to H3 tri-methylated on lysine 9 (H3K9me3) stimulates the acetyltransferase activity of Tip60.

H3K9me3 is a well-studied chromatin PTM that is generally associated with heterochromatin and silenced regions of the genome. It is thought that deposition of H3K9me3 constitutes a major pathway in the establishment and maintenance of heterochromatin[9]. This interpretation is consistent with accumulation of this histone modification at repetitive sequences, such as satellite repeats, which constitute pericentromeric (constitutive) heterochromatin. Nevertheless, additional H3K9 methylation spots of unknown function are found in actively transcribed genes.

Chromodomains are short sequence motifs found in several chromatin factors, which have been implicated in the recognition of lysine methylation. For example, the chromodomains of heterochromatin protein 1 (HP1) and Polycomb have been shown to have affinity for H3K9me3 and H3K27me3, respectively. While these interactions are usually used to explain the co-distribution of the binding proteins with their cognate histone modifications on chromatin, the work of Sun *et al.* introduces a different and highly interesting concept. H3K9me3 is not required for the recruitment of Tip60 to sites of DNA damage. Instead, the interaction of Tip60/ATM with the MRN complex is sufficient for chromatin localization. However, the interaction with H3K9me3 is essential for Tip60 HAT stimulation and the initiation of downstream DSB repair events. Although the regulation of enzymatic activity of chromatin modifying enzymes by pre-existing

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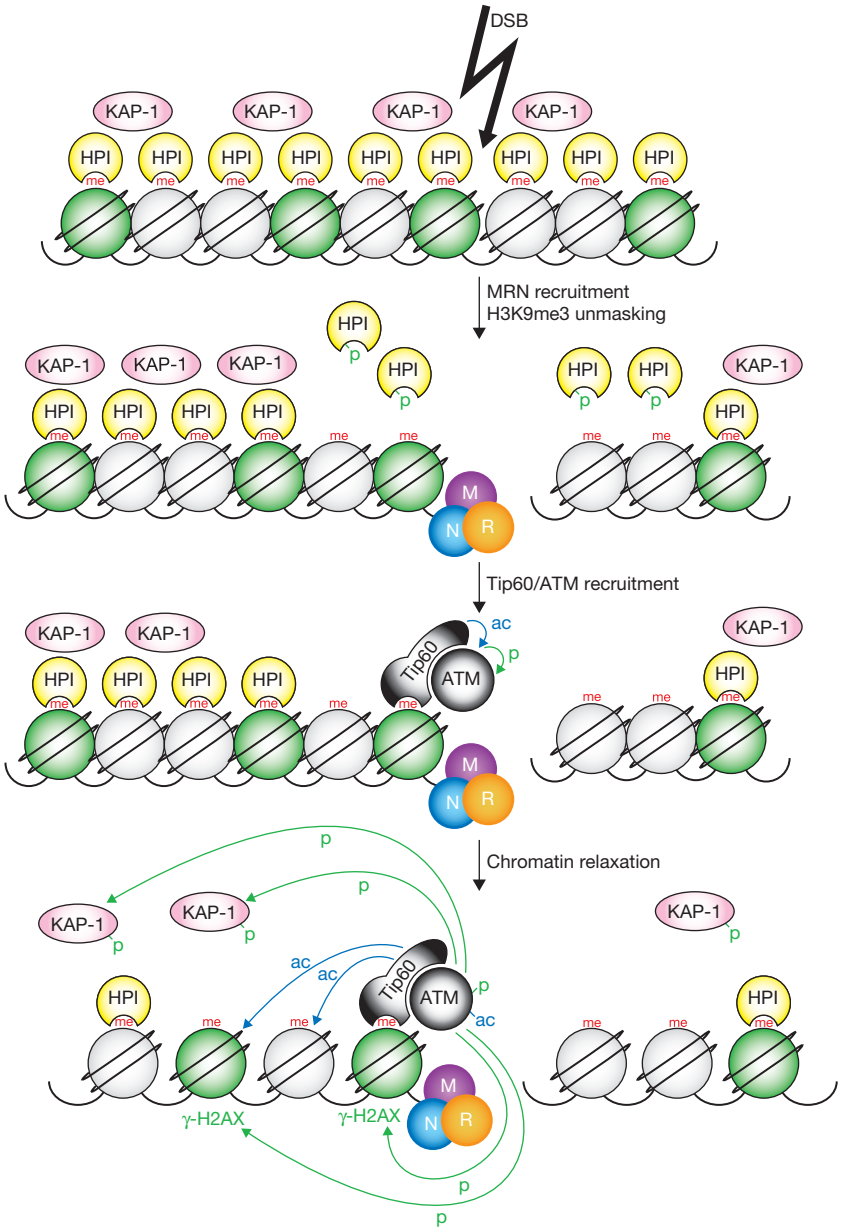

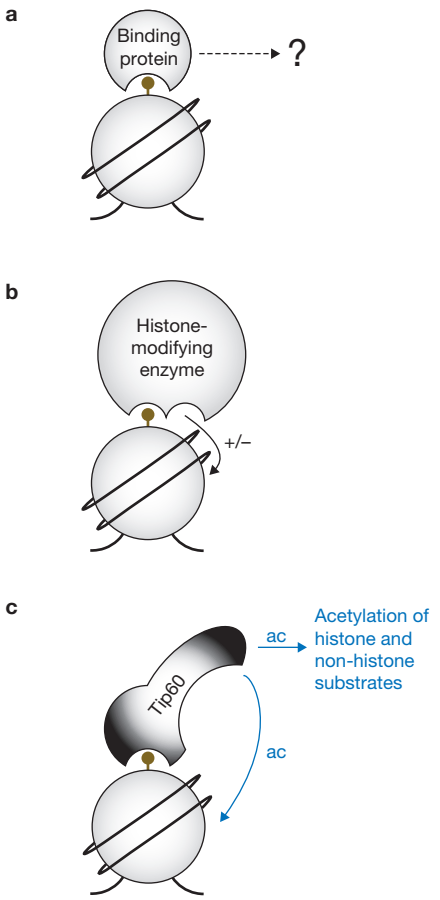

Figure 1 Proximal events of DSB recognition and repair in heterochromatin. In response to DSBs, unknown mechanisms activate casein kinase 2 (not shown), which phosphorylates HP1, resulting in unmasking of the H3K9me3 heterochromatin modification. The DSB site-binding MRN complex (MRE11–RAD50– NBS1) recruits Tip60/ATM. Binding of the Tip60 chromodomain to H3K9me3 stimulates HAT activity of Tip60, initiating activation of ATM kinase by acetylation-induced autophosphorylation. KAP-1 and H2AX are immediate targets of ATM phosphorylation activity. Together with the hyperacetylation of H3 and H4, the phosphorylation of KAP-1 and H2AX contributes to chromatin relaxation and the progression of downstream DSB repair. For clarity, only events occurring at one break site are given in detail. Nucleosomes containing the histone variant H2AX are in green, and those containing canonical H2A are in grey. PTM: ac, acetylation (blue); me, methylation (red); p, phosphorylation (green).

Figure 2 Readout and translation of histone PTM. (**a**) The downstream working mechanisms of many histone PTM-binding proteins (such as HP1) are unclear. (**b**) The activity of histone-modifying enzymes is modulated by pre-existing patterns of histone PTMs. (**c**) As discovered by Sun *et al.*, binding of the Tip60 chromodomain to H3K9me3 stimulates the distinct histone acetyltransferase domain, which also has important non-histone substrates such as ATM[2]. PTM: ac, acetylation (blue); brown dots represent any histone PTM on chromatin.

modifications has been observed before, the findings of Sun *et al.* add a new dimension. Binding of a histone modification at one domain of Tip60 stimulates (presumably allosterically) the enzymatic activity at a different region of the protein. Obviously, elucidating the exact molecular mechanisms of chromodomain and HAT region crosstalk will be highly interesting not only for understanding DSBs repair, but also for the readout and translation of histone modifications.

Given that H3K9me3 binds to and activates Tip60, how specifically is this particular histone PTM involved in DSB repair? Two possibilities can be considered: *de novo* establishment of H3K9me3 specifically at sites of DSBs, or selective uncovering of pre-existing H3K9me3 modification at sites of DSBs. Depletion of HP1 is sufficient to support activation of Tip60 at sites of DSBs[2]. Also, prolonged and artificial chromatin recruitment of MRN components or ATM can initiate H2AX phosphorylation[10]. Potentially, these results suggest that unmasking of previously occupied H3K9me3 is the trigger. This interpretation is consistent with DSB-induced phosphorylation of the HP1 chromodomain by casein kinase 2 (CK2), which causes the release of phosphorylated HP1 from chromatin early in the repair pathway[11]. Although it was also

shown that HP1 is specifically recruited to sites of DSBs[12], this might be a later event, independent of the HP1 chromodomain and/or H3K9me3 interaction. An initial burst of HP1 modification and release from chromatin, thereby exposing H3K9me3, might be all that is needed to initiate the DSB repair cascade. A similar mechanism of pre-existing histone PTMs being exposed by chromatin changes has been suggested for H3K79me/H4K20me-dependent localization/recruitment of 53BP1 later in the DSB repair process[1].

Considering that H3K9me3 unmasking occurs parallel to MRN-mediated Tip60/ATM recruitment, and as an early step in a DSB repair pathway, this mechanism probably takes place in regions of heterochromatin where H3K9me3 is readily found. Differences in the biology of euchromatin and heterochromatin have long been studied. Whereas euchromatin is open for transcription and early replicating during S phase, heterochromatic regions are more densely packed, mostly transcriptionally silent and are late replicating in S phase[9]. Overall, the barrier imposed by chromatin to the DNA repair process might be even higher in heterochromatin. Therefore, DSB repair pathways dedicated to heterochromatic DNA might have evolved.

In euchromatin, ATM signalling is dispensable for repair, as DSB factors have free access to their target regions. In contrast, in heterochromatin ATM is required, as repair factors are unable to access the DSBs[13]. An important enzymatic target of ATM activity therefore is KAP-1, a transcriptional co-repressor and HP1-interacting protein. KAP-1 phosphorylation seems to render heterochromatin more plastic and accessible[14]. In agreement with a role of the H3K9me3/Tip60/ATM activation pathway for DSB repair in heterochromatin, ATM can be activated by changes in chromatin structure, for example, by exposing cells to hypotonic stress[6]; this is expected to loosen chromatin structure, thereby putatively exposing H3K9me3. Interestingly, genetic work in *Drosophila melanogaster* demonstrated that the H3K9me3-establishing enzyme Su(var)3-9 is essential for heterochromatic genome stability, as flies mutant in this factor have elevated frequencies of spontaneous DNA damage in heterochromatin[15].

The new work raises important questions for the understanding of DSB repair, as well as for the working mechanisms of histone modifications. Is this pathway only relevant to heterochromatin, or are similar mechanisms at play in euchromatin? What exactly triggers the exposure of H3K9me3 at DSBs? What molecular rearrangements are required to allosterically activate the HAT domain of Tip60? Are there other enzymes that translate histone modifications in trans by modifying non-histone substrates? Clearly, more remains to be learnt about the role of histone PTMs in DSBs and other biological pathways.

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TGFβ helps cells fly solo

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Intravital imaging demonstrates that TGFβ signalling regulates the mode of cancer cell motility. Cells with active TGFβ signalling migrate as single cells and are capable of hematogenous and lymphatic spread, whereas cells lacking TGFβ signalling invade lymphatics collectively.

Migration is a key characteristic of malignant tumour cells and an essential step in the metastatic cascade. Whereas many tumour cells seem to migrate with a mesenchymal phenotype, others migrate as chains or groups that maintain epithelial characteristics. On page 1287 of this issue, Sahai and colleagues use elegant intravital imaging studies to visualize either collective or single cell migration of cancer cells[1]. These two migratory phenotypes are regulated by many factors, but the authors show that the TGFβ/Smad signalling axis can drive a switch to single cell migration, and that the mode of migration determines the way that tumours spread to secondary sites.

Numerous *in vitro* and *in vivo* studies have correlated the abilty of a cell to migrate with a mesenchymal phenotype and increased metastatic potential. It is well established that single tumour cells are capable of either mesenchymal or amoeboid migration and doubts have been raised as to the relative importance of mesenchymal migration. Mesenchymal- and amoeboid-migrating cells share common characteristics, including non-epithelial cell morphology, loss of cell–cell contacts and the presence of actin stress fibres[2]. A key difference is that proteolytic extracellular matrix degradation occurs during mesenchymal migration only. Single cell mesenchymal migration has been identified in numerous

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