Chromatin and DNA repair: the benefits of relaxation

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DNA is tightly wrapped around histones to form chromatin. How DNA repair molecules interact with this chromatin structure is an emerging question. New findings suggest that chromatin structure impedes the access of DNA repair proteins to sites of DNA damage, thus establishing a mechanism for the function of chromatin remodelling complexes during DNA repair.

Chromatin is a dynamic structure that can adopt markedly different conformations. For example, interphase and mitotic chromatin display contrasting degrees of compaction. It is now well established that during transcription, different conformations of chromatin act as important regulatory switches. A 'compact' chromatin structure generally inhibits the binding of transcriptional regulators, as well as the process of RNA polymerase elongation[1]. As a strategy to counteract this inhibitory effect, cells employ molecular machines that consume the energy of ATP to remodel the nucleosomal structure. The binding and activity of these remodelling complexes can, in turn, be regulated by post-translational modifications that occur on histones in response to transcriptional activators or repressors bound at promoter regions.

Until recently, the functional interplay between chromatin structure and the critical process of DNA repair has remained poorly understood, in part because it is difficult to untangle the confounding effects of chromatin structure on transcription from a direct impact on DNA repair. However, numerous recent studies have implicated chromatin-remodelling complexes and histone-modifying activities in DNA double-strand break (DSB) repair (reviewed in refs 2, 3). These studies have been particularly fruitful in budding yeast, where a single DSB can be generated at a defined locus in a controlled fashion.

Recent evidence in yeast indicates that histone acetyltransferase (HAT) complexes and other chromatin remodelling factors are specifically recruited to DSBs[4-8] where they are hypothesized to participate in the remodelling of chromatin at the locus surrounding the DSB, possibly to stimulate DNA repair. Correspondingly, cells deficient in *ESA1*, the yeast TIP60 homologue (a HAT), or human cells that overexpress a catalytically inactive TIP60 mutant, are sensitive to DSB-producing agents[5,9]. However, it is unclear how the chromatin changes mediated by these histone and chromatin modifiers are linked to the activity of DNA repair and the presence of checkpoint proteins at DSBs. On page 91 of this issue, Murr *et al.* provide evidence to support the hypothesis that chromatin structure acts as a barrier to the recruitment of DNA repair proteins and DNA damage signalling proteins at sites of DNA damage[10]. The inference from this work is that histone-modifying and chromatin-remodelling complexes act in concert to pry open this barrier to allow DNA repair to proceed.

To examine the relationship between DNA repair and chromatin remodelling in mammalian cells, the authors specifically addressed the role of mammalian TRRAP in this process through the generation of a conditional knockout of the *TRRAP* gene in murine cells and by RNA interference technology in human cells. TRRAP is a core component of a number of HAT complexes, including the TIP60 complex. Murr *et al.* observed that the selective ablation of *TRRAP* resulted in a significant DSB repair defect. Importantly, the authors show specifically that repair by homologous recombination is defective in *trrap*$^{-/-}$ murine cells by using a clever system that harnesses the site-specific I-*Sce*I meganuclease to deliver a single DSB at a reporter cassette[11]. In addition to providing a system to monitor homologous recombination, this experimental scheme allowed the authors to monitor histone modification and protein recruitment at the locus surrounding the I-*Sce*I site concomitant with the kinetics of DSB repair. They found that the repair defect observed in *trrap*$^{-/-}$ cells correlated with a decrease in TIP60 recruitment and H4 acetylation at DNA breaks, consistent with a role for TRRAP as a component of the TIP60 complex.

While accumulation of γ-H2AX (a phosphorylated form of the H2AX histone variant induced by DSBs) and activation of the ATM-dependent DNA damage signalling pathway both appear intact, the authors find that recruitment of RAD51 (a key homologous recombination protein) to I-*Sce*I-induced DSBs is compromised in *TRRAP*-deficient cells. Moreover, they find decreased formation of subnuclear foci containing RAD51, BRCA1 (also a key homologous recombination protein) and 53BP1 (a DNA damage signalling protein) after exposure to ionizing irradiation. Intriguingly, these results indicate that *TRRAP*-dependent acetylation at DSB sites is only required for a subset of the DNA damage response.

The compelling link between histone H4 hypoacetylation, defective recruitment of repair factors and compromised DSB repair in *TRRAP*-deficient cells suggests that the accessibility of the DSB was impeded in these cells. Remarkably, treatment of *TRRAP*-deficient cells with hypotonic shock, sodium butyrate or chloroquine — agents known to relax chromatin structure — resulted in a near-complete rescue of defects in RAD51 focus formation and subsequent homologous recombination repair. Together, these data suggest that TRRAP is necessary for TIP60 retention at sites of DNA damage, and that TIP60-mediated histone acetylation leads to the chromatin relaxation required for efficient localization of a subset of repair proteins necessary for homologous recombination. Importantly, as not all DSB repair or signalling proteins appear to be affected by chromatin structure, we can infer that chromatin, instead of being a general or non-specific inhibitor of DSB repair, may act as a selective barrier that regulates a subset of the DNA damage response. If this hypothesis is substantiated by future studies, it raises the important question of what event initiates the recruitment of these histone-modifying and chromatin-remodelling activities to sites of damage.

Two possibilities exist: either the chromatin remodelling machinery can detect DNA lesions independently of DSB repair or signalling (perhaps as a consequence of chromatin perturbations induced by the DNA lesion itself), or remodeller recruitment is a downstream step in

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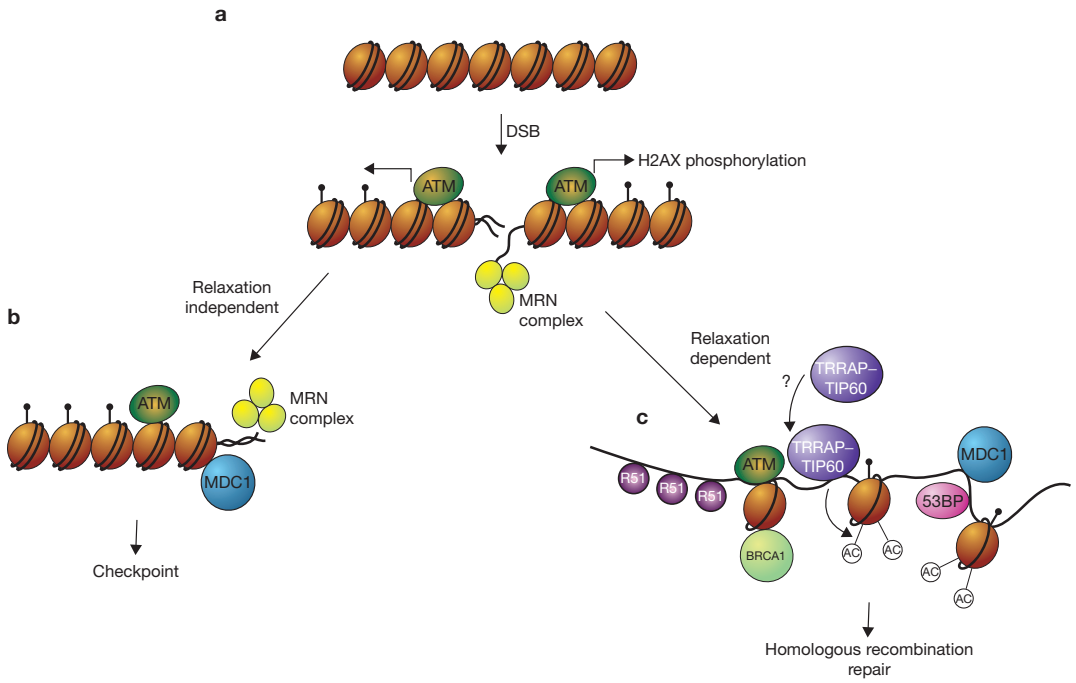

Figure 1 Chromatin-dependent and -independent responses to DNA damage. (**a**) In this model, chromatin acts as a selective barrier that impedes the recruitment of a subset of DNA double-strand break (DSB) proteins. (**b**) In response to a DSB, a chromatin-independent pathway involving proteins such as ATM, MDC1 and the MRE11–Rad50–NBS1 (MRN) complex is activated after their recruitment to the site of DNA damage. ATM activation leads to phosphorylation of H2AX to yield γ-H2AX (a critical histone modification), as well as activation of the DNA damage checkpoint pathway. (**c**) The accumulation of γ-H2AX or the association of MRN to DSBs, in turn, stimulates the recruitment of chromatin remodelling activities such as the TRRAP–TIP60 complex. As a consequence, TIP60-dependent chromatin relaxation allows for the recruitment of RAD51 and BRCA1 and stimulates DSB repair through homologous recombination.

the DNA damage signalling cascade. As Murr *et al.* show that Mdc1 and Nbs1 recruitment to nuclear foci are insensitive to chromatin conformation, and as formation of γ-H2AX is required for the recruitment (or maintenance) of proteins at sites of DNA damage (possibly including the homologue of the TRRAP–TIP60 complex in yeast, NuA4; ref. 4), we favour the second possibility (Fig. 1). This is also supported by the recent direct demonstration that chromatin remodelling at a single DSB event in *Saccharomyces cerevisiae* is dependent on the activity of the Mre11–Rad50–Xrs2 complex[12], the yeast orthologue of the mammalian Mre11–Rad50–Nbs1 complex and a proposed sensor of DSBs. Thus, the response to DSBs may branch out into chromatin-state-sensitive and -insensitive pathways after the initial detection of the lesion (Fig. 1).

Why establish such a mechanism? One possibility may be to channel the DNA lesion into a specific DSB repair process. Indeed, we note that Murr *et al.* found that BRCA1 and RAD51 are sensitive to *TRRAP* dosage. TRRAP-dependent TIP60 recruitment and subsequent chromatin relaxation may act as a switch to target a lesion into the homologous recombination repair pathway. Supporting this possibility, DSB resection (a key step in homologous recombination) is likely regulated by chromatin structure and chromatin remodelling[8]. It will be interesting to test whether similar or different remodelling activities are required for non-homologous end-joining, the second major pathway of DSB repair in mammals.

Finally, the requirement for chromatin relaxation during DSB repair raises an additional interesting question: is the active reversal of histone modifications (such as histone H4 acetylation) required for the removal of DSB repair and signalling proteins from sites of DNA damage after DNA repair? Such an activity could be critical; for example, to allow cells to re-enter the cell cycle after DNA repair. Indeed, in budding yeast, cell cycle re-entry after DNA repair requires the desphosphorylation of histone H2A by a newly identified phosphatase complex[13], suggesting that chromatin structure has a critical function in this process. It is tempting to speculate that the termination of checkpoint signalling may also require activities such as histone deacetylases or the active exchange of modified histones with their unmodified counterparts[14]. Nevertheless, the suggestion that chromatin relaxation has an important function in the response to DSBs suggests that the same basic mechanisms are employed by HAT complexes to regulate transcription and DNA repair. The work of Murr *et al.* underscores the point that chromatin relaxation is an important part of a larger response to DNA damage. □

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