KDM5A demethylase: Erasing histone modifications to promote repair of DNA breaks

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Repairing DNA breaks within the complexity of the cell chromatin is challenging. In this issue, Gong et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201611135) identify the histone demethylase KDM5A as a critical editor of the cells' "histone code" that is required to recruit DNA repair complexes to DNA breaks.

DNA double-strand breaks (DSBs) are potentially lethal DNA lesions that can arise through collapse of replication forks or exposure to genotoxic agents. DSBs are primarily repaired through two mechanisms: nonhomologous end-joining (NHEJ) and homologous recombination. For NHEJ, DNA is processed to remove damaged bases and the ends religated, which can lead to small insertions or deletions. Homologous recombination, which operates in S-G2M, uses homologous DNA on sister chromatids as a template for repair and has higher fidelity than NHEJ. DSB repair is initiated by a defined signal transduction pathway in which DNA breaks activate the ATM and/or DNA-PKcs kinases. This initiates a cascade of phosphorylation events, including phosphorylation of histone variant H2AX, and loading of multiple repair proteins, including the 53BP1 protein and BRCA1 complexes, onto the chromatin.

DSB repair is also critically dependent on the local chromatin structure in which the DSB arises (Gursoy-Yuzugullu et al., 2016). Chromatin is made up of nucleosomes, which contain 147 bp of DNA wrapped around a histone core. The N-terminal tails of histones extend outward from the nucleosome and can be modified through acetylation and methylation, thereby creating binding sites for reader proteins that recognize histone modifications. Histone modification and chromatin binding proteins regulate the extent of chromatin compaction, transcription, and other DNA transactions. It is now clear that the repair of DSBs is also directly coupled to the machinery that regulates chromatin and nucleosome reorganization (Gursoy-Yuzugullu et al., 2016). For example, the Smerdon laboratory (Smerdon and Lieberman, 1978) demonstrated that UV-damaged sites undergo rapid nucleosome reorganization, and the Jeggo laboratory (Goodarzi et al., 2008) showed that DSB repair in heterochromatin required the ATM kinase and chromatin reorganization to promote access to damaged DNA. Chromatin remodeling during DSB repair can include eviction of nucleosomes to create nucleosome-free regions for repair, deployment of chromatin remolding ATPases to move nucleosomes or exchange histone variants, and dynamic changes in histone methylation and acetylation (Smeenk and van Attikum, 2013; Gursoy-Yuzugullu et al., 2016). This has led to the idea that DSB repair requires chromatin remodeling to create open, mobile chromatin structures that facilitate access to and repair of the DSB (Smeenk and van Attikum, 2013). Further, DSBs arising in different chromatin structures (e.g., genes versus heterochromatin) require different types of remodeling events and histone modifications to allow access to individual DSBs. However, although much work has focused on how increases in histone acetylation and methylation during repair regulate DSB repair, there has been little investigation of how preexisting histone methylation marks may inhibit repair. Therefore, the study in this issue by Gong et al. is timely as it demonstrates that removal of preexisting H3K4me3 by KDM5A is required to promote recruitment of a second repair protein, ZMYND8, to DSBs.

Previous work from this and other laboratories demonstrated that the chromatin binding protein ZMYND8 is recruited to DSBs (Gong et al., 2015; Spruijt et al., 2016). ZMYND8 contains a coupled PHD-BRD-PWWP domain that may read multiple histone modifications, including acetylated histone H4 (H4ac) and H3 (H3K14ac) and methylated H3 (H3K4me1; Gong et al., 2015; Savitsky et al., 2016; Spruijt et al., 2016). ZMYND8 is recruited to H4ac sites at DSBs and corecruits the NuRD complex, which contains two key proteins—a CHD3/CHD4 ATPase for nucleosome remodeling and an HDAC for histone deacetylation. Importantly, the ZMYND8–NuRD complex is only recruited to DSBs near genes and plays a key role in suppressing local transcription during DSB repair. Silencing local transcription is important for preventing uncontrolled interactions between RNA pol II complexes and the DSB repair machinery. Although recruitment of ZMYND8 requires H4 acetylation by the Tip60 acetyltransferase, which is increased at DSBs (Xu et al., 2010), other work suggested that the PHD-BRD-PWWP domain of ZMYND8 is a multifunctional reader that recognizes combinations of H3K4me and H3K14ac (Savitsky et al., 2016). Further, because H3K4me3 can block ZMYND8–NuRD loading, it is unclear how ZMYND8 can be loaded onto DSBs in genes, which contain a high density of H3K4me3. In their new study, Gong et al. (2017) demonstrate that the histone demethylase KDM5A is recruited to DSBs, removes H3K4me3, and promotes binding of ZMYND8 at DSBs.

KDM5A is a H3K4 histone demethylase identified by the authors from a screen of chromatin proteins that are both recruited to DSBs and required for recruiting ZMYND8.

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Targeting KDM5A (but not other KDMs) with siRNA blocked recruitment of ZMYND8 (and ZMYND8's binding partner, the NuRD complex) to DSBs. This placed KMD5A upstream of ZMYND8 in the damage response. Because KDM5A demethylates H3K4me3, Gong et al. (2017) assessed whether H3K4me3 was reduced after DNA damage. They used an established system in which induction of the AsiSI nuclease creates multiple DSBs (>80; Aymard et al., 2014), followed by chromatin immunoprecipitation and DNA sequencing to map H3K4me3. They found demethylation of H3K4me3 adjacent to DSBs, but not at gene promoters far from the breaks, demonstrating specific demethylation of H3K4me3 at genes adjacent to DSBs. The decrease in H3K4me3 was reduced (but not completely blocked) using siRNA or inhibitors of KDM5A. KDM5A recruitment to DSBs therefore leads to rapid demethylation of H3K4me3, although a role for additional KDMs in H3K4me3 demethylation cannot be fully excluded.

Next, Gong et al. (2017) examined how KDM5A regulates ZMYND8 recruitment. Using a series of peptides, they demonstrated that binding of ZMYND8 to the H3 tail was blocked by H3K4me3, but not by either unmodified H3 or H3K9me3. Interestingly, NuRD and ZMYND8 could both bind to mono- and dimethylated H3 (H3K4me1 and H3K4me2), indicating exquisite specificity for inhibition of ZMYND8 binding by H3K4me3. The researchers then followed this by examining which domains of KDM5A mediated recruitment to DSBs. KDM5A, like most KDMs, contains several PHD motifs, which may bind methylated lysines, as well the catalytic jmjN and jmjC domains. Mutational analysis revealed that PHD1 is required to recruit KDM5A to DSBs, whereas both the PHD1 motif and KDM5A's catalytic activity are required to load ZMYND8. This demonstrated that demethylation of H3K4me3 is upstream of, and required for, recruitment of ZMYND8. Finally, depletion of other proteins required to recruit ZMYND8, including Tip60 (which acetylates histone H4), the CHD4 subunit of NuRD, and ZMY ND8 itself, did not affect KDM5A recruitment, confirming that KDM5A recruitment is upstream of ZMYND8 and recruited to DSBs independently of ZMYND8.

Finally, Gong et al. (2017) addressed the question of how KDM5A was recruited to DSBs. ZMYND8 recruitment was previously shown to require the poly-ADP ribose polymerase (PARP) family of proteins (Smeenk and van Attikum, 2013; Savitsky et al., 2016; Spruijt et al., 2016). PARPs can create PAR chains at sites of DNA damage, which function as stress signals to recruit chromatin regulatory proteins to DSBs. Gong et al. (2017) showed that PARP1 activity is required to recruit KDM5A and, using live imaging, demonstrated that KDM5A is recruited to DSBs before ZMYND8. KDM5A recruitment to PAR chains at damaged chromatin therefore leads to rapid erasure of H3K4me3, removing the inhibitory H3K4 methylation and promoting recruitment of the ZMYND8–NuRD complex. A further interesting aspect of this work is the role H3K4me3 plays in regulating transcription. H3K4me3 is largely restricted to active genes and several studies, including from the authors (Aymard et al., 2014; Gong et al., 2015), have indicated that DNA breaks near active genes can lead to gene silencing. By creating a DSB in a reporter gene or by visualizing transcriptionally active chromatin domains, Gong et al. (2017) noted that loss of KDM5A attenuates the normal drop in local transcriptional activity adjacent to DSBs, consistent with loading of ZMYND8–NuRD (and loss of H3K4me3) acting as a general transcriptional repressor. This underlines the importance of H3K4me3 demethylation during DSB repair to repress transcription.

This paper provides new insight into how histone modifications are linked to DSB repair. The observation that H3K4me3 must be demethylated to allow for repair to proceed, and that this process is essential for limiting local transcription, indicates that rewriting of the underlying histone signature at specific sites is an important aspect of DSB repair. The removal of inhibitory methylation marks is therefore just as important as the addition of new histone modifications. Further, because H3K4 demethylation is restricted to genes, this indicates that repair mechanisms are precisely coupled with the functional domain in which the DSB arises, with distinct chromatin structures requiring specialized histone and chromatin processing before repair can start. Remodeling of distinct local chromatin structures to create a common chromatin template for the DNA repair machinery may be the driving principle here.

There are several interesting areas to be pursued based on this work. If H3K4me3 blocks ZMYND8–NuRD loading at genes, it will be interesting to determine why ZMYND8–NuRD is not recruited to DSBs in regions of the chromatin that lack the H3K4me3 modification. A potential explanation is that removal of H3K4me3 plus other histone modifications defines binding ZMYND8, and that these other modifications may be restricted to genes. Mapping unique histone modifications at DSBs in genes compared with other chromatin structures will be important for addressing this. In a similar vein, PARP recruitment and PARylation, which recruit KDM5A, are ubiquitous. It is therefore possible that KDM5A is recruited broadly to all sites of damage. Specificity for KDM5A at genes might be achieved through several mechanisms. PAR may recruit KDM5A to all sites, but it is only retained at DSBs where H3K4me3 is also present. The PHD1 of KDM5A is required to retain it at DSBs, and it is possible that KDM5A can detect both PAR (that has poorly defined binding domains) as well as H3K4me3. A further area to explore is how ZMYND8 and NuRD interact with histone marks at DSBs. Both ZMYND8 and NuRD subunits, including CHD4, contain multiple histone reader modules, and it is still unclear which reader modules interact with which modifications during DSB repair (Gong et al., 2015; Savitsky et al., 2016; Spruijt et al., 2016). Perhaps reader modules/histone modification combinations that are used during DSB repair are distinct from those used during other cellular functions of ZMYND8. Further, ZMYND8 has multiple splice variants, which may contribute to variation in ZMYND8 binding specificity. More work will be needed to precisely identify which domains and reader functions of ZMYND8 are required for its DNA repair functions. Finally, as Gong et al. (2017) noted, many of the proteins involved in ZMYND8 recruitment, including KDMs, ZMYND8, and NuRD, are disrupted in cancers. This may indicate a specific repair defect in these tumors that can be exploited for therapeutic gain. Elucidating the distinct role of chromatin in genomic stability and DNA repair will continue to provide new insight into the tight coupling of these processes in cells.

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