# Molecular Biology Select

The basic unit of chromatin is the nucleosome—about 146 basepairs of DNA wrapped around an octamer of histone proteins. A number of different posttranslational modifications to histones, such as methylation or acetylation, regulate access to the DNA, allowing vital processes including transcription and DNA repair to take place. In this Molecular Biology Select, I discuss recent findings that reveal how certain histone modifications can be reversed and how some of these epigenetic marks depend on one another. The emerging picture is that even a stable, heritable chromatin state may include some remarkably dynamic and reversible features.

Demethylation Demystified

The lysine residues of histone proteins can be mono-, di-, or trimethylated, signaling either transcriptional activation or repression depending on which lysine residue is methylated and to what extent. Histone methylation was considered a stable modification until the recent discovery that an amine oxidase called LSD1/BHC110 is able to demethylate the lysine 4 of histone H3. Now, Tsukada et al. describe a new family of histone demethylases containing the so-called JmjC domain as their signature motif. They purified JHDM1 (for JmjC domain containing histone demethylase 1) from extracts of HeLa cells and demonstrated in vitro that JHDM1 specifically demethylates lysine 36 of histone H3 with a preference for the dimethylated form. Dimethylated lysine 36 also may be the relevant substrate in vivo given that overexpression of JHDM1 in cells results in a decrease in dimethylated lysine 36 but not in the mono- or trimethylated forms. These findings are important for several reasons. The JmjC family of oxidases is larger than the LSD1 family and could include dedicated enzymes that reverse the wide variety of known histone methylation marks. In addition, proteins containing JmjC motifs, unlike LSD1, are present in budding yeast. In fact, the budding yeast JHDM1 homolog also demethylates lysine 36 of H3, establishing that histone demethylation is likely to be a general feature of eukaryotic chromatin. Finally, unlike LSD1, the JmjC-dependent reaction mechanism does not require a protonated amino group intermediate. Thus, JmjC enzymes could, at least in principle, target trimethylated lysines, such as the characteristic heterochromatin marks on lysine 9 or lysine 27 of histone H3. It remains to be seen whether any of the JmjC oxidases are specific for trimethylated lysines.

Methylation of lysine 36 occurs in actively transcribed genes and helps to prevent spurious transcription from within open reading frames. Future studies should reveal whether lysine 36 demethylation controls transcription and whether crosstalk exists between JHDM1-mediated demethylation and other repressive histone modifications. *Y.I. Tsukada et al. (2005). Nature. doi:10.1038/nature04433.*

Polycomb Proteins and DNA Methyltransferases Get Hitched
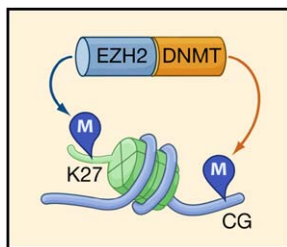
The Polycomb Group protein EZH2 directly controls DNA methylation. The amino-terminal tail of histone H3 protrudes from a nucleosome (green) and lysine residue K27 is methylated (blue balloon) by EZH2. This enzyme directly contacts DNA methyltransferases (DNMT), leading to methylation of cytosine on nearby DNA (orange arrow).

Stable heritable gene silencing is associated with several chromatin marks. These include methylation of histone H3 on lysine 27 (mediated by a histone methyltransferase and the Polycomb Group protein EZH2) and methylation of DNA (mediated by one of the DNA methyltransferases). In their new study, Vire et al. provide the first glimpse of a direct connection between these two key repressive epigenetic pathways. They find that EZH2 physically interacts with all known active DNA methyltransferases in vitro and in vivo. EZH2 recruits DNA methyltransferases to the promoters of known EZH2 target genes, and depletion of either EZH2 or the DNA methyltransferases leads to reactivation of these genes. Interestingly, DNA methylation at EZH2 target gene promoters is decreased in cells with reduced EZH2, whereas EZH2 overexpression increases DNA methylation. These findings establish a functional connection between Polycomb-dependent histone methylation and DNA methylation. It will be interesting to see whether this relationship is a common feature of genes that are silenced by a Polycomb-dependent mechanism. This work could shed new light on our understanding of epigenetic memory, X-chromosome inactivation, and pathological situations such as cancer.

The results also highlight the dynamic nature of DNA methylation, raising the question of how methylated DNA disappears after EZH2 depletion. Methylated DNA could simply be "diluted" during successive rounds of DNA replication. Alternatively, a DNA demethylase may actively remove the methyl groups. Bacterial oxidases from the AlkB family are known to remove methyl groups from DNA as part of a DNA-repair process that reverses alkylation damage. However, mammalian DNA-demethylating enzymes have not been identified so far. Perhaps DNA methylation is another "stable" epigenetic mark waiting to be toppled by a demethylase. *E. Vire et al. (2005). Nature. doi:10.1038/nature04431.*

Methylation and Ubiquitination—A Polycomb Connection?

Polycomb group proteins exist in at least two functionally distinct complexes: the EZH2 (PRC2) complex, which methylates histone H3 on lysine 27, and the PRC1 complex, which ubiquitinates histone H2A on lysine 119. Both complexes contribute to heritable gene silencing. The PRC1 complex contains several proteins with a ring domain, a motif characteristic of a class of E3 ubiquitin ligases. The RING1B protein is known to have catalytic activity, but the function of two other ring-domain proteins, RING1A and BMI1, in the ubiquitination of histone H2A is less clear. In their new work, Cao et al. reconstitute the human PRC1 complex and demonstrate that the RING1A and BMI1 proteins have no E3 ligase activity on their own but do stimulate RING1B activity. Using embryonic fibroblasts from mice deficient in RING1A and BMI1, the authors establish that RING1A and BMI1 are required for ubiquitination of H2A at *Hox* gene promoters and for silencing of *Hox* genes, which are key developmental regulators controlled by Polycomb proteins. Interestingly, the EZH2 complex and methylation of H3 on lysine 27 are still seen at *Hox* gene promoters in mouse cells that lack BMI1. Conversely, decreasing lysine 27 methylation by reducing the expression of an EZH2 complex component reduces PRC1 recruitment and H2A ubiquitination. In a related study, Hernandez-Munoz et al. report that components of the EZH2 complex also are required for proper recruitment of BMI1 to heterochromatin domains. Intriguingly, they observe that the DNA methyltransferase DNMT1 is required for proper BMI1 localization but not for EZH2-dependent methylation of lysine 27. Considering these findings together with those of Vire et al., it is tempting to speculate that DNA methylation acts as an intermediary to establish sequential recruitment of the two Polycomb-protein complexes (EZH2 and PRC1) in mammalian cells. This recruitment order fits nicely with the finding in vitro that a protein in the PRC1 complex binds specifically to lysine 27 that has been methylated by EZH2.

These studies raise many interesting questions. BMI1 has received considerable attention as it has been implicated in the renewal capability of stem cells and in tumor formation. The next step will be to test whether these functions depend on H2A ubiquitination. It also remains a mystery how H2A ubiquitination contributes to gene silencing mechanistically.

*R. Cao et al. (2005). Mol. Cell **20**, 845–854.*

*I. Hernandez-Munoz et al. (2005). Mol. Cell Biol. **25**, 11047–11058.*

γ-H2AX: What Goes on, Must Come off

Modification of histones is also a key event in the response of cells to DNA damage. Phosphorylation of the histone variant H2AX on serine 139, resulting in so-called γ-H2AX, is one of the earliest events after DNA damage and marks the chromatin surrounding the damaged site. In budding yeast, which lacks a H2AX variant, the regular histone H2A is phosphorylated in response to DNA damage. Two recent papers identify the phosphatases that counteract phosphorylation of H2AX in mammalian cells and H2A in yeast. Keogh et al. tested budding yeast mutants lacking phosphoserine phosphatases and found that only loss of the Pph3 phosphatase led to an increase in phosphorylated H2A. As part of a three-protein complex called HTP-C (for histone H2A phosphatase complex), Pph3 dephosphorylates phospho-H2A both in vitro and in vivo. Interestingly, the kinetics of phospho-H2A appearance and decline at sites of DNA damage is unchanged in HTP-C mutant cells, suggesting that phospho-H2A is first removed from chromatin—possibly with the help of chromatin remodeling complexes—before it is dephosphorylated by HTP-C. Whereas normal cells resume proliferation after a temporary checkpoint-induced cell-cycle arrest, HTP-C mutant yeast show prolonged checkpoint activation and a delayed recovery from cell-cycle arrest. This checkpoint recovery defect is abolished in yeast carrying a mutation in the H2A phosphorylation site and so is likely to be due to an excess of phospho-H2A. In a related study, Chowdhury et al. identify PP2A as the counterpart of Pph3 in cultured human cell lines. Cells with reduced PP2A activity show stronger γ-H2AX immunofluorescence signals in so-called repair foci that assemble at sites of DNA damage. This result and the observation that PP2A is recruited to repair foci in a γ-H2AX-dependent manner seem most consistent with the interpretation that γ-H2AX dephosphorylation occurs on chromatin in contrast to the finding reported by Keogh et al. for yeast, where phospho-H2A is first removed from chromatin before being dephosphorylated. However, further investigation will be necessary to establish where exactly mammalian H2AX is dephosphorylated. One complication is that PP2A phosphatase activity is involved in the regulation of many different cellular processes. In particular, PP2A prevents activation of the ATM kinase, a central player in the response to DNA damage and one of the kinases that can phosphorylate H2AX. In principle, hyperactivation of ATM by PP2A could account for the increase in γ-H2AX. However, the authors establish nicely that γ-H2AX still increases after inhibition of PP2A in ATM-deficient cells, arguing that γ-H2AX dephosphorylation by PP2A is independent of ATM activation. Once it becomes possible to dissect the various roles of PP2A in DNA repair, the investigators can elucidate whether, as in yeast, dephosphorylation of γ-H2AX promotes recovery from cell-cycle arrest induced by DNA damage.

M.C. Keogh et al. (2005). Nature. doi:10.1038/nature04384.

*D. Chowdhury et al. (2005). Mol Cell. **20**, 801–809.*

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