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H3K27 Demethylases, at Long Last

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Methylation of lysine 27 on histone H3 (H3K27me) by the Polycomb complex (PRC2) proteins is associated with gene silencing in many developmental processes. A cluster of recent papers (Agger et al., 2007; De Santa et al., 2007; Lan et al., 2007; Lee et al., 2007) identify the JmjC-domain proteins UTX and JMJD3 as H3K27-specific demethylases that remove this methyl mark, enabling the activation of genes involved in animal body patterning and the inflammatory response.

The methylation of histone proteins at specific residues plays a major role in the maintenance of active and silent states of gene expression in developmental processes. An elegant example is provided by regulation of *Hox* genes, whose expression domains are established during body formation by early signaling events. These genes are subsequently maintained in the active or silent state by the Trithorax and Polycomb methyltransferases, respectively (reviewed in Iimura and Pourquie, 2007; Schuettengruber et al., 2007). However, to allow commitment and differentiation to different lineages, the regulatory system controlling developmental events must retain plasticity and have the ability to rapidly switch expression states in a noise-resistant manner in response to certain developmental and environmental cues. To accomplish this, efficient mechanisms for the removal of histone methylation must exist. The discovery of several histone demethylases has now been reported, including the amine oxidase LSD1 and Jumonji C (JmjC) domain proteins (reviewed in Shi and Whetstine, 2007; Klose and Zhang, 2007). To allow for precise regulation, histone demethylases target specific lysine residues and methylation states (mono-, di-, or trimethyl). Recent discoveries include enzymes that specifically demethylate distinct methyl-lysine residues (H3K4me3, H3K9me3, and H3K36me3) on histone H3 (Shi and Whetstine, 2007; Klose and Zhang, 2007). Now, four groups (Agger et al., 2007; De Santa et al., 2007; Lan et al., 2007; Lee et al., 2007) report the discovery of UTX and JMJD3, two JmjC-domain proteins that specifically demethylate di- and trimethyl-lysine 27 (H3K27me2/3) on histone H3. These exciting findings have broad implications for how Polycomb-mediated silencing can be counteracted to enable changes in cell fate.

Trimethylation of H3K27 Marks Silent Genes

An enzymatic subunit of the Polycomb PRC2 complex, EZH2, methylates H3K27 to mediate gene silencing (Figure 1A; Schuettengruber et al., 2007). Although originally identified as *Hox* repressors, PRC2-complex proteins occupy many developmentally important genes and are involved in mammalian X chromosome inactivation, germline development, stem cell identity, cell-cycle regulation, and cancer (Schuettengruber et al., 2007). H3K27me3 contributes to the recruitment and/or stabilization of the Polycomb PRC1 complex on chromatin, which in turn mediates gene silencing (Schuettengruber et al., 2007). Genomic analyses suggest that the presence of H3K27me3 at transcriptional start sites is generally correlated with the repression of gene expression, even at target genes containing a high level of H3K4me3, a modification associated with transcriptional initiation by RNA polymerase II (reviewed in Bernstein et al., 2007). Chromatin regions containing both H3K4me3 and H3K27me3 have been termed "bivalent domains" and are enriched in embryonic stem (ES) cells at promoters of genes encoding key developmental transcription factors and components of critical signaling pathways (Mikkelsen et al., 2007). Upon ES cell differentiation, many bivalent domains resolve into transcriptionally active H3K4me3 or transcriptionally silent H3K27me3 chromatin regions, depending on the specific cell fate and lineage commitment, and some remain bivalent in terminally differentiated cells (Mikkelsen et al., 2007). One consequence of the rapid transition from the bivalent to the active state during differentiation is a need for removal of H3K27me3, implying the existence of H3K27me3 demethylases.

JMJD3 and UTX Are H3K27me3 Demethylases

The recently discovered H3K27 demethylases, UTX and JMJD3, contain highly homologous JmjC domains; another member of the same evolutionarily conserved JmjC subfamily is UTY. In addition to the JmjC domain, both UTX and UTY, but not JMJD3, contain tetratricopeptide motifs, predicted to mediate protein-protein interactions. In vitro, UTX and JMJD3 catalyze transition of H3K27me3 and H3K27me2 to H3K27me1 on bulk histone substrates, with H3K27me3 being a preferred substrate (Agger et al., 2007; De Santa et al., 2007; Lan et al., 2007; Lee et al., 2007; see Figure 1A). In the same assay conditions, UTY was enzymatically inactive (Lan et al., 2007), suggesting either that it requires the presence of additional cofactors for its enzymatic activity or, alternatively, that it is not an active enzyme but rather has demethylase-independent functions. In

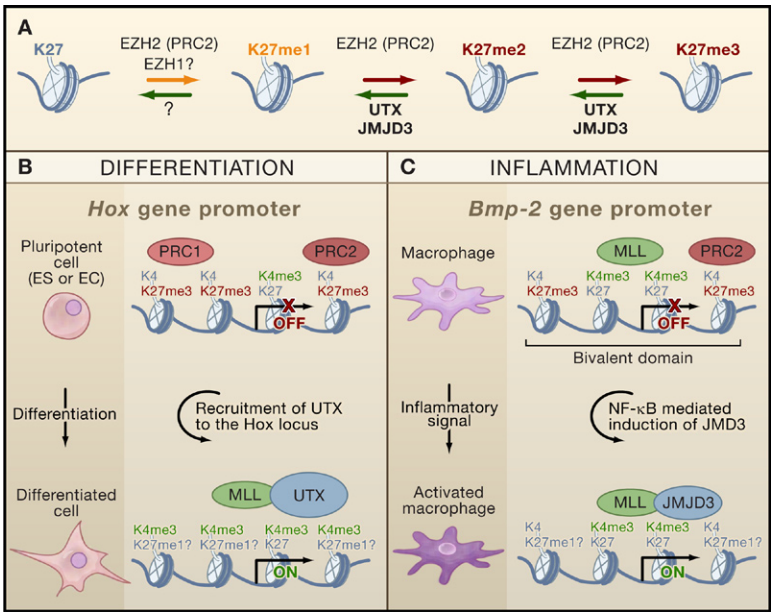

Figure 1. Methylation and Demethylation of H3K27

(A) An enzymatic subunit of the Polycomb complex PRC2 called EZH2 catalyzes mono-, di-, and trimethylation of H3K27. UTX and JMJD3 are specific H3K27 di- and trimethyl demethylases.

(B) UTX demethylates *Hox* gene promoters during the transition from a pluripotent to a differentiated state. ES, embryonic stem cell; EC, embryonic carcinoma cell.

(C) JMJD3 is induced upon activation of macrophages by inflammatory stimuli. In macrophage activation, the enzyme has the capacity to resolve bivalent domains, for example at the promoter of *Bmp-2*, to enhance expression of target genes.

contrast to bulk histones, activity of the recombinant UTX and JMJD3 on the nucleosomal substrates is very weak, indicating that they require protein cofactors for chromatin recognition (Lan et al., 2007).

The activity of UTX or JMJD3 might result in the concomitant increase in H3K27me1 at target genes. It is not known whether an enzyme exists to remove H3K27 monomethylation, and none of the recent studies addressed the status of H3K27me1 at UTX and JMJD3 target loci. Interestingly, recent genomic analysis revealed an unexpected result: in contrast to H3K27me3, which marks silent loci, H3K27me1 is enriched downstream from transcription start sites of active genes (Barski et al., 2007). Could this be a signature of UTX/JMJD3 present at these genes counteracting methylation by the PRC2 complex?

UTX Regulates *Hox* Gene Expression

In pluripotent cells, *Hox* genes remain silent and are marked by a continuous pattern of H3K27me3. During vertebrate embryogenesis, *Hox* genes exhibit temporal and spatial colinearity of expression, with the most anterior *Hox* genes activated first and in the more anterior body structures, and the more posterior *Hox* genes activated later and in the more posterior body structures (Iimura and Pourquie, 2007). Remarkably, the colinearity of *Hox* gene expression is preserved during differentiation of ES or human embryonic carcinoma NT2/D1 cells with retinoic acid. Histone methylation patterns demarcate the expression boundary: active genes within a cluster are marked by a continuous stretch of H3K4me2/3, whereas silent genes are marked by H3K27me3. Are H3K27me3 demethylases involved in a switch from an H3K27me3 to H3K4me3 pattern of marking during activation of *Hox* genes? Agger et al. (2007) and Lee et al. (2007) demonstrate that upon differentiation of NT2/D1 cells with retinoic acid, UTX is recruited to the promoters of the anterior genes of the *HOXA* and *B* loci. Recruitment of UTX to these promoters coincides with disappearance of the H3K27me3, decreased occupancy of the PRC2 complex components SUZ12 and EZH2, and gene activation (Figure 1B). Importantly, knockdown of UTX expression suppresses H3K27 demethylation and decreases *HOXB1* induction upon retinoic acid treatment (Agger et al., 2007).

As mentioned above, *Hox* gene methylation patterns are unique in that H3K4me2/3 is not confined to the transcriptional start sites, as is the case for most genes; instead, active regions of the *Hox* cluster are marked by long, continuous H3K4me2/3 stretches devoid of H3K27me3 (Bernstein et al., 2007). Is UTX (or another H3K27 demethylase) demethylating H3K27 throughout this long stretch? De Santa et al. (2007) show that JMJD3 is transiently associated with the *HoxA7* and *HoxA11* loci during bone marrow cell differentiation, suggesting that multiple H3K27 demethylases control expression of *Hox* gene clusters. Do H3K27 demethylases transiently occupy intergenic and coding regions at the time of *Hox* activation during early differentiation? These questions become particularly important in the context of the findings reported by Lan et al. (2007). Genomic analysis across the four *HOX* clusters in primary human fibroblasts revealed that UTX occupancy is restricted to a narrow region directly downstream of the transcription start sites, independent of their transcriptional status. This is in striking contrast with the broad patterns of H3K4me2 and H3K27me3, which divide the loci into two large domains: one co-occupied by H3K4me2 and polymerase II, and one co-occupied by H3K27me3 and PRC2. UTX binding correlates with diminished levels of H3K27me3 and PRC2 at transcription start sites. However, within the domain marked by H3K4me2 and polymerase II, levels of H3K27me3 and PRC2 at coding and intergenic regions remain low despite the lack of UTX binding (Lan et al., 2007). Consistent with these findings, Lee et al. (2007) observe in human embryonic kidney (HEK) 293 cells that UTX enrichment at *HOXA13* and

HOXC4 loci is much higher at the promoter region as compared to the coding region. UTX knockdown leads to increased levels of H3K27me3 and both Polycomb complexes PRC1 and PRC2 at transcriptional start sites of these genes (Figure 1B). Thus, in a differentiated cell type, human fibroblasts, UTX controls steady-state levels of H3K27me3 at transcription start sites, but not at coding and intergenic regions of *HOX* loci. Moreover, the steady state of H3K27me3 at transcription start sites is a result of a dynamic equilibrium between the opposing functions of UTX and PRC2, determined by a local concentration of these enzymes at a target gene.

JMJD3 and Resolution of Bivalent Domains

How do signaling pathways connect to the chromatin-modifying machinery to regulate cellular processes? Work by De Santa et al. (2007) demonstrates that JMJD3 is rapidly induced in macrophages in response to an inflammatory stimulus, lipopolysaccharide (LPS). This induction is directly dependent on NF-κB, a major regulator of the inflammatory response. Accumulation of JMJD3 during macrophage activation affects expression of a subset of genes induced by inflammation including *Bmp-2*, a known target of silencing by Polycomb. BMP-2 protein is a secreted morphogen involved in bone formation and embryonic development; its expression is induced during macrophage activation with LPS, perhaps to facilitate bone healing. Prior to activation, the *Bmp-2* promoter is bivalently marked (Figure 1C). Upon treatment with LPS, H3K27me3 levels at the *Bmp-2* locus decrease in a JMJD3-dependent manner, whereas H3K4me3 levels remain unchanged, consistent with a resolution of the bivalent domain into an active state (Figure 1C).

UTX and JMJD3 in Development

Involvement of H3K27 demethylases in *Hox* gene regulation and resolution of bivalent domains suggests important roles for these enzymes in regulating development. Lan et al. (2007) present loss-of-function studies of one of the two UTX homologs encoded by the zebrafish genome. Morpholino-mediated zUTX1 knockdown results in truncation of the posterior notochord and abnormalities in posterior structures of the embryo. Analysis of *Hox* expression patterns in zUTX1 morphants reveals a posterior shift of *Hox* expression domains, consistent with its role in counteracting Polycomb silencing and reminiscent of the *trithorax* group phenotypes. Although detailed analysis of the zUTX2 phenotype is not provided, the authors indicate that zUTX2 loss of function also affects posterior development. It is curious that zebrafish UTX seems to preferentially affect expression of posterior *Hox* genes, even though UTX is involved in anterior *Hox* regulation during the differentiation of human embryonic carcinoma cells. It is also not entirely clear that certain aspects of the reported zUTX1 phenotype, most notably notochord truncation, are a direct result of abnormal *Hox* gene expression. Rather, it is likely that an earlier developmental event involved in formation of the dorsal mesoderm also requires UTX activity.

The *C. elegans* genome encodes one UTX-like protein and three JMJD3-like proteins. Agger et al. (2007) show that deletion of one of the three JMJD3 homologs in *C. elegans* (XJ193) leads to abnormal gonad migration and accumulation of cells arrested in meiosis at diakinesis, indicating that H3K27 demethylation is involved in direct or indirect regulation of a crucial checkpoint in oocyte maturation. In sum, reports by Lan et al. (2007) and Agger et al. (2007) give us a taste of the complex roles that H3K27 demethylases might play in developmental processes. Given that so many developmentally important genes are silenced by Polycomb, there is little doubt that additional functions of H3K27 demethylases in regulation of development of multicellular organisms will be soon uncovered.

UTX and JMJD3 Associate with H3K4 Methyltransferase Complexes

H3K27me3 and H3K4me3 have opposing roles in gene regulation. Indeed, active versus silent states of gene expression might be determined by the relative levels of H3K4me3 and H3K27me3 at transcription start sites. Interestingly, UTX was recently reported to associate with two H3K4 methyltransferases of the MLL family, MLL3 and MLL4 (also called MLL2 and ALR), suggesting cooperation between H3K4 methylation and H3K27 demethylation (Issaeva et al., 2007, Cho et al., 2007). MLL-family proteins specifically catalyze H3K4 methylation and associate with the so-called core complex components RbBP5, WDR5, and ASH2, which are all essential for H3K4me3 on nucleosomal substrates (reviewed in Ruthenburg et al., 2007). Consistent with earlier findings, Lee et al. (2007) show that UTX is present in a large protein complex containing MLL3/4, core complex proteins, and H3K4 methyltransferase activity. In parallel, De Santa et al. (2007) report that JMJD3 coimmunoprecipitates with the core complex component RbBP5, suggesting that association of H3K27 demethylases with H3K4 methyltransferases may be a general phenomenon. The physical association between H3K27 demethylases and H3K4 methyltransferases raises the issue of how the bivalent domains are protected from precocious demethylation. One plausible explanation is that the association of UTX/JMJD3 with MLL family members is regulated by cellular signaling. Another possibility is that high levels of the PRC2 complex present in ES cells shift the equilibrium toward H3K27 methylation.

H3K27 Demethylation and a Multistable-Switch Model of Epigenetic Memory

Discovery of H3K27 demethylases forces us to rethink the nature of epigenetic memory. It has been a prevalent assumption that epigenetic memory relies on chemical stability of methylation marks at individual nucleosomes. Indeed, even after the discovery of histone demethylases, it was suggested that some methylation marks, in particular H3K27me3, might be more stable than others and therefore function as true epigenetic signals (Trojer and Reinberg, 2006). The existence of H3K27 demethylases is difficult to reconcile with static mod-

els of epigenetic inheritance. However, does this suggest that histone methylation cannot convey epigenetic memory? Certainly not. In fact, the discovery of H3K27 demethylases provides a strong boost to the dynamic models of epigenetic memory that rely on the concept of multistability. Bi- or multistable systems can exist in one of two or more alternative states under the same conditions; the current state of the system is determined by its immediate history. Multistable processes exhibit a property known as "hysteresis," the ability of the system to remain in a newly acquired state even after an external stimulus that initiated the switch is diminished. Many epigenetic processes involving H3K27 methylation indeed appear to be regulated this way: in processes such as X inactivation or *Hox* gene expression, transient signaling events establish one of the alternative regulatory states, and, once established, such states can be maintained and propagated even in the absence of the initial signal.

The most definitive treatment of the dynamic model of epigenetic memory has been provided by illuminating theoretical work (Dodd et al., 2007). Dodd and coworkers demonstrate that robust bistability arises even in simplified mathematical models of chromatin. Their model relies on the existence of modifying and demodifying enzymes, such as methyltransferases and demethylases, and provides several nontrivial predictions regarding the nature of chromatin modifications and epigenetic memory, including a remarkable resistance to the noise resulting from random partitioning of modified nucleosomes upon replication. However, producing bistability in this model requires cooperativity, or the ability of modified nucleosomes to stimulate addition of the same modification on nearby nucleosomes. Moreover, bistability is enhanced by the ability of modified nucleosomes to stimulate removal of competing modifications. How might regulation of the epigenetic state by opposing functions of H3K4 and H3K27 methylation fit into this model? H3K4 methyltransferases contain protein domains known or predicted to preferentially recognize H3K4me, such as WDR5 or H3K4me-binding PHD fingers (Ruthenburg et al., 2007), suggesting the existence of a positive feedback loop. At least a subset of H3K4 methyltransferases associate with H3K27 demethylases and thus are able to stimulate removal of the competing modification. However, it remains to be established whether the PRC2 complex stimulates addition of H3K27me directly or indirectly and whether it associates with H3K4 demethylases.

Although it will be challenging to test, the multistable-switch model of epigenetic memory is an attractive one: it allows for robust and noise-resistant switching of the epigenetic state in response to developmental and environmental cues. Sufficiently severe transient alteration of equilibrium between opposing chromatin-modifying activities might allow the system to cross the barrier from one state to another. Such alteration can be introduced by known signaling pathways. One striking example is provided by nuclear reprogramming. The transient introduction of specific transcription factors creates sufficient disequilibrium at loci controlling pluripotency to flip the epigenetic switch (Okita et al., 2007; Wernig et al., 2007). Whether H3K27 demethylases are recruited to the pluripotency loci in nuclear reprogramming remains an open question.

The discovery of H3K27 demethylases sheds new light on the intricacies of gene expression and advances our understanding of how silencing by Polycomb can be counteracted in developmental processes. These findings have broad implications for how we envisage the mechanisms underlying epigenetic memory and offer us a glimpse into an exciting world of dynamic chromatin.

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