Role of H3K27 Demethylases Jmjd3 and UTX in Transcriptional Regulation

M.R. HÜBNER AND D.L. SPECTOR

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Correspondence: huebner@cshl.edu; spector@cshl.edu

The influence of histone amino-terminal covalent modifications on gene regulation has drawn intense research efforts in recent years. It is now clear that activating and inactivating modifications have a key role in determining the gene-expression profile of an individual cell or cell lineage. Thus, differences in these modifications have a pivotal role in determining and maintaining cell fate during development. The interplay of histone methyltransferases (HMTs) and demethylases confers the plasticity necessary for changes in the gene-expression profile of a cell during differentiation or changes following environmental cues. The histone H3 lysine 27 (H3K27) demethylases Jmjd3 and UTX remove the gene-inactivating H3K27 dimethyl and trimethyl marks and are involved in inducing and/or maintaining gene expression. In this chapter, we highlight the role of the H3K27 demethylases Jmjd3 and UTX in gene expression.

Transcriptional repression of individual genes, which is established as a result of a developmental process or cellular differentiation, is generally maintained throughout the lifetime of a cell. Importantly, these gene-expression patterns are propagated to the daughter cells, thereby providing a cellular memory for the differentiation decisions made in their progenitors. For instance, the expression patterns of *HOX* genes are established during early body-plan formation and maintained in the active or inactive state by Trithorax and Polycomb proteins, respectively (for review, see Schuettengruber et al. 2007; Schwartz and Pirrotta 2007). Many of these gene-expression patterns are maintained for several decades in the case of animals or even for centuries in some plants. However, to allow plasticity during a developmental program or in response to environmental cues, gene silencing needs to be reversible.

Gene silencing by the Polycomb group (PcG) of proteins involves trimethylation of lysine 27 in the amino-terminal tail of histone H3 and is involved in mammalian X-chromosome inactivation, stem cell identity, and germline development (Plath et al. 2003; Zhao et al. 2008; Schuettengruber and Cavalli 2009). The enzyme to catalyze H3K27 trimethylation in differentiated cells is Ezh2 (enhancer of zeste homolog 2), which, together with its essential cofactor EED as well as SUZ12 and RbAp48, are part of the Polycomb repressive complex 2 (PRC2) (for review, see Simon and Kingston 2009). The Ezh2 homolog Ezh1 forms a distinct PRC2 complex that maintains H3K27me3 modification and repression of developmental target genes (Margueron et al. 2008; Shen et al. 2008). However, Ezh1 is present at lower levels than Ezh2 in actively dividing cells and has a weaker methyltransferase activity (Margueron et al. 2008). H3K27me3 is recognized by the chromo domain of the Polycomb protein in *Drosophila* or its orthologs in

mammalian cells. Polycomb, together with Bmi1 and the H2A ubiquityl ligase RING1B, is a member of PRC1, which mediates chromatin compaction and, ultimately, gene silencing (Francis et al. 2004).

The closely related Jmjd3 (Kdm6B [jumonji-domain-containing protein 3]) and UTX (KDM6A [a ubiquitously transcribed tetratricopeptide repeat gene on the X chromosome]) are members of a growing number of Fe(II) and α -ketoglutarate-dependent demethylases containing a conserved Jumonji C (JmjC) domain (the *jumonji* gene was named for a mutation in mice that causes abnormal cruciform neural grooves (in Japanese, *jumonji* means cruciform) (see Fig. 1) (for review, see Klose et al. 2006; Lan et al. 2008; Mosammaparast and Shi 2010). To date, 30 JmjC-domain-containing proteins are known in human and mouse, most of which possess demethylase activity to a number of different histone and nonhistone substrates (Huang and Berger 2008). Only LSD1/KDM1a, which balances promoter H3K4/H3K9 methylation for activation or repression of its target genes, and its homolog LSD2/KDM1b, which modulates intragenic H3K4 dimethylation, do not contain a JmjC domain (Shi et al. 2004; Klose and Zhang 2007; Shi and Whetstone 2007; Cloos et al. 2008; Fang et al. 2010). Both Jmjd3 and UTX have been shown to demethylate H3K27me2/3 (Agger et al. 2007; De Santa et al. 2007; Hong et al. 2007; Lan et al. 2007; Lee et al. 2007). UTX is located at Xp11.2 on the X chromosome, escapes X-chromosome inactivation, and is ubiquitously expressed (Greenfield et al. 1998). UTX and its male homolog UTY, which shares 83% amino acid identity (Greenfield et al. 1998), but not Jmjd3, contain tetratricopeptide repeats in their amino-terminal region that are assumed to mediate protein-protein interactions (Blatch and Lassle 1999). All three proteins have a carboxy-terminal treble-clef zinc finger.

Figure 1. Protein domains of human Jmjd3 and UTX. (Dark gray) Catalytic JmjC domain (horizontal), (light gray) tetrapeptide repeats (vertical). Indicated also are the Fe(II) binding site and zinc finger. Numbers indicate amino acid positions.

H3K27 DEMETHYLASES IN DIFFERENTIATION

In undifferentiated ES cells, the promoter of many key developmental genes is marked by H3K27me3 (Boyer et al. 2006; Bracken et al. 2006; Lee et al. 2006; Pan et al. 2007). These promoters often carry the activating H3K4 mark as well and are thus called “bivalent” (Bernstein et al. 2006; Mikkelsen et al. 2007; Pan et al. 2007; Zhao et al. 2007; Ku et al. 2008). The presence of the H3K27me3 modification keeps these developmental regulators repressed or expressed at very low levels and is believed to keep them poised for rapid activation once the appropriate signal is received and the repressive mark is removed (Bernstein et al. 2006). At the end of ES cell lineage commitment, these bivalent domains have been resolved to carry either activating (H3K4me3) or inactivating (H3K27me3) marks in a lineage-specific manner (Bernstein et al. 2006). In ES cells, the pluripotency genes *OCT4*, *SOX2*, and *NANOG* are rich in trimethylated H3 lysine 4 (H3K4me3), which is generally associated with active genes. These genes acquire the H3K27me3 mark as they get repressed during differentiation (Pan et al. 2007).

During embryonic development, Nodal signaling activates Smad2 and Smad3, which get recruited to H3K27 trimethylated and PcG-silenced target genes (Dahle et al. 2010). Smad2/3 then recruit Jmjd3, which leads to the loss of H3K27me3, dissociation of PRC2 components, and ultimately, derepression of target genes, e.g., the *Brachyury* locus in ES cells (Dahle et al. 2010).

Jmjd3, but not UTX, is required for neural commitment in the differentiation of mouse ES cells into neural stem cells in adherent monocultures (Burgold et al. 2008). Together with four other JmjC genes (*Jmjd2b*, *Jarid1a*, *Jarid1c*, and *Phf8*), Jmjd3 is up-regulated in differentiating neural precursors at day 8 of differentiation but down-regulated in self-renewing neural stem cells at day 26 (Burgold et al. 2008). Jmjd3 gets recruited to the transcriptional start site of *Pax6* (a homeodomain transcription factor), *Nestin* (a neurofilament protein), and *Sox1* (a homeodomain protein with a key role in neural commitment), and depletion of the Jmjd3 protein leads to decreased up-regulation of these genes (Burgold et al. 2008). Jmjd3 resolves the bivalent status of the *Nestin* promoter by demethylating H3K27 and thus activating Nestin expression, which is a key regulator and marker of neurogenesis (Burgold et al. 2008). In contrast, the initial recruitment of Jmjd3 to the

Pax6 transcriptional start site does not result in decreased H3K27me3 levels. This indicates that *Pax6* can be expressed in the presence of H3K27me3 and the demethylase activity of Jmjd3 might be regulated and context specific. Similarly, genome-wide studies showed that certain genes can be expressed despite H3K27me3 in their promoter region (Ringrose and Paro 2004; Bracken et al. 2006; Papp and Muller 2006; Roh et al. 2006). Following Jmjd3 depletion, *Pax6* up-regulation is impaired, indicating that Jmjd3 contributes to *Pax6* expression through a mechanism that is independent of its H3K27 demethylase activity or that the involvement of Jmjd3 is indirect (Burgold et al. 2008).

During neural differentiation, global H3K27me3 levels, as well as levels of H3K9me3 and H4K20me3, increase (Akizu et al. 2010), and a number of genes become repressed by the H3K27me3-dependent PcG machinery (Boyer et al. 2006; Lee et al. 2006; Pietersen and van Lohuizen 2008). Interestingly, the maintenance of global H3K27me3 levels is not required for neural differentiation or neuroblast proliferation (Akizu et al. 2010). However, in the developing spinal cord, dorsal interneuron generation is regulated by H3K27me3-dependent repression of the *Noggin* promoter. Noggin is an extracellular inhibitor of bone morphogenetic proteins (BMPs), which are essential for the formation of dorsal neural cell types (Liu and Niswander 2005). In turn, BMPs activate Noggin expression, thereby creating a negative regulatory feedback loop. Noggin activation by BMP is mediated by Smad1/4-dependent H3K27 demethylation of the *Noggin* promoter by Jmjd3 (Akizu et al. 2010).

As a result of retinoic acid (RA)-induced neuronal differentiation in P19 cells, Jmjd3 expression, but not UTX, increases and has a role in activating the *Mash1/Ascl1* gene through Hes1-dependent binding to the *Mash1* promoter (Dai et al. 2010). Similarly, Jmjd3 is involved in the RA- or Notch-dependent induction of differentiation along the neuronal pathway and subsequent forebrain development in the mouse (Jepsen et al. 2007). In the absence of ligand, RA-dependent differentiation along the neuronal pathway is repressed by the nuclear receptor corepressor 2 (NCoR2, also known as SMRT). SMRT acts by repressing the expression of Jmjd3, which prevents the up-regulation of genes involved in neuronal differentiation. Conversely, the *Jmjd3* gene is a direct target of the liganded RA receptor, which causes the up-regulation of Jmjd3 during RA-induced neural differentiation (Jepsen

et al. 2007). Similarly, overexpression of *Jmjd3*, but not a catalytically inactive mutant, results in the induction of neuronal genes (Jepsen et al. 2007). The opposing functions of SMRT and the RA receptor are mediated by their competitive binding to the canonical RARE (retinoic acid response element) region in the *Jmjd3* promoter region (Jepsen et al. 2007).

Jmjd3 is the only JmjC-domain-containing protein that is up-regulated by lipopolysaccharide (LPS) stimulation and transdifferentiation of mouse macrophages. This up-regulation depends on the binding of NF- κ B to conserved NF- κ B-binding sites located in the promoter region just upstream of the transcription start site of the *Jmjd3* gene in macrophages (De Santa et al. 2007). Conversely, the *Jmjd3* paralog UTX, which shares 84% of sequence similarity in the JmjC domain (Hong et al. 2007), is expressed at low and constant levels. *Jmjd3* is involved in up-regulating the *Bmp-2* gene by reducing H3K27me3 levels in mouse macrophages 8–24 h postinduction with LPS (De Santa et al. 2007). Similarly, the *HOXA11* and *Irf4* genes are *Jmjd3* targets in differentiating bone marrow cells (De Santa et al. 2007; Satoh et al. 2010). *Jmjd3* is in a complex with the H3K4 HMT MLL/Trx2 (but not MLL1) as well as with the MLL complex components Wdr5, RbBP5, and Ash2L (De Santa et al. 2007). Conversely, UTX was found to bind to MLL2/ALR (Issaeva et al. 2007). This suggests that gene expression can be facilitated by a combination in the same complex of enzymes that add the activating H3K4me3 mark and remove the repressive H3K27me3 modification. Indeed, *Jmjd3* gets recruited to >70% of LPS-induced genes, where it preferentially binds to promoters that carry high levels of H3K4me3 (De Santa et al. 2009). More specifically, *Jmjd3* binds to a region within 2.5 kb of the transcriptional start site, and its binding peaks after 2 h of LPS stimulation. Interestingly, most of the *Jmjd3* target genes are not associated with H3K27me3. Furthermore, the *Jmjd3* target genes that had H3K27 methylation before stimulation did not show a statistically significant decrease in H3K27 methylation after stimulation (De Santa et al. 2009). The deletion of *Jmjd3* did not affect the expression of most of its target genes, suggesting redundancy and/or additional functions of *Jmjd3*. However, in a few hundred *Jmjd3* target genes, polymerase II (Pol II) recruitment and transcription were moderately impaired (De Santa et al. 2009). Furthermore, overall levels of H3K27me1–3, as well as that of H3K4me1–3 in fetal-liver-derived macrophages from wild-type and *Jmjd3*^{−/−} mice, were identical, indicating that H3K27 methylation does not undergo continuous turnover (De Santa et al. 2009).

The hematopoietic T-box transcription factor T-bet has a role in the differentiation of naïve CD4⁺ T cells into the T-helper type-1 (Th1) lineage. The differentiation of CD4⁺ T cells into the Th1 lineage is accompanied by H3K27 demethylation and H3K4me2 modification of the *interferon- γ* (*Ifn- γ*) gene, whereas differentiation into the Th2 lineage is accompanied by silencing of the *Ifn- γ* locus by the H3K27me3 mark (Schoenborn et al. 2007). The activation of the *Ifn- γ* locus is mediated by direct interaction of T-bet with the H3K27 demethylase *Jmjd3* and the H3K4 HMT Set7/9 (Lewis et al. 2007; Miller et al. 2008). Fur-

thermore, *Jmjd3* is involved in the transcriptional activation of specific marker genes of alternatively activated (M2) macrophages (Ishii et al. 2009).

Stem cell self-renewal and differentiation are balanced in the human epidermis, which has a turnover rate of ~4 weeks and undergoes continual replenishment from stem cells of the inner epidermal basal layer. Concomitant with differentiation, these cells exit the cell cycle, detach from the basal membrane, and migrate outward (for review, see Blanpain and Fuchs 2006). Genes involved in differentiation are suppressed in undifferentiated cells and induced during epidermal differentiation. This process involves the *Jmjd3*-dependent loss of H3K27me3 at the promoter regions as well as SUZ12 binding to the promoters of the induced genes (Sen et al. 2008). Consequently, overexpression of *Jmjd3* or depletion of SUZ12 or Ezh2 leads to premature epidermal differentiation, whereas depletion of *Jmjd3* prevents epidermal differentiation (Sen et al. 2008). Another example for the reprogramming of skin cells is the induction of a large repair transcriptome during wound healing in murine skin repair, which is associated with a loss of Polycomb-mediated gene silencing and an up-regulation of *Jmjd3* and UTX (Shaw and Martin 2009).

UTX mediates the demethylation of H3K27me3 of the muscle-specific myogenin (*Myog*) and muscle creatine kinase (*CKm*) genes during myogenesis (Seenundun et al. 2010). Demethylation is initially mediated through UTX binding to the homeobox transactivator Six4, which leads to loss of H3K27me3 in the region upstream of the transcription start site (Seenundun et al. 2010). Subsequent H3K27 demethylation in the coding region requires elongating RNA Pol II (Seenundun et al. 2010), and *Drosophila* UTX has been shown to directly associate with Ser-2-phosphorylated elongating Pol II on actively transcribed genes (Smith et al. 2008).

REGULATION OF *HOX* GENES BY H3K27 DEMETHYLASES

RA-induced differentiation of human pluripotent embryonic NT2/D1 teratocarcinoma cells into neural lineages is associated with UTX binding to *HOXA1–3* and *HOXB1–3* genes and subsequent H3K4 methylation, H3K27 demethylation, and loss of PRC2 binding (Agger et al. 2007; Lee et al. 2007). Interestingly, the recruitment of Ash2L and H3K4 trimethylation occurred 18 h post-RA administration, whereas UTX binding and H3K27 demethylation took 24 hours (Lee et al. 2007). This suggests that the interplay of these opposing activities regulates the transcriptional activation of *HOX* gene promoters. Consequently, disruption of the *Caenorhabditis elegans* *Jmjd3* homolog XJ193 led to defects in gonadal development and organization (Agger et al. 2007). Similarly, in HEK293 cells, UTX was shown to bind to the *HOX* gene cluster in a complex with MLL3/4 (ALR), which leads to demethylation of H3K27me2/3, H3K4 methylation, and the loss of PRC1-mediated transcriptional repression (Lee et al. 2007) (please note that in this chapter, we use the nomenclature of Glaser et al. 2006: MLL2/Trx2 and MLL4/ALR. In contrast, some publications designate

MLL4 as Trx2, whereas MLL2 is designated ALR; e.g., see De Santa et al. 2007; Issaeva et al. 2007; Lee et al. 2007). Depletion of UTX leads to an increased binding of Ring-finger-containing proteins of the PRC1 complex, Bmi1 and Ring1A, and an increase in H2A monoubiquitylation of *HOXA13* and *HOXC4* (Lee et al. 2007). However, the depletion of UTX and the concomitant increase in H3K27 methylation did not have a significant effect on the occupancy of the *HOXA13* and *HOXC4* promoters with components of the MLL3/4 (ALR) complex or the methylation status of H3K4 (Lee et al. 2007). Furthermore, UTX was shown to regulate H3K27 methylation in some but not all *HOXD* genes in HeLa cells (Lan et al. 2007). In particular, H3K27me3 levels of the promoter region of *HOXD10–12* were elevated following UTX knock-down (Lan et al. 2007). Additionally, the 3' end of *HOXD12* had a higher H3K27me3 level in UTX-depleted cells. In contrast, Jmjd3 depletion had little effect on the H3K27me3 status of the *HOXD* cluster (Lan et al. 2007). In primary human fibroblasts, 90% of all UTX-binding sites were in *HOX* loci, independent of their expression status, and concentrated in relatively narrow regions within 500 bp downstream from the transcriptional start site of *HOX* genes (Lan et al. 2007). In contrast, UTX was entirely excluded from the *HOX* loci in mouse ES cells where *HOX* genes are transcriptionally silent and associated with H3K27me3 (Lan et al. 2007). Inhibition of the UTX homolog in zebrafish results in misregulation of *HOX* genes and developmental defects of the posterior trunk (Lan et al. 2007).

H3K27 DEMETHYLASES IN SENESENCE

Replicative senescence in human multipotent stem cells is associated with the decreased expression of histone deacetylase 1 and 2 (HDAC1/2) followed by down-regulation of the PcG proteins Ezh2, SUZ12, and Bmi1 and the up-regulation of Jmjd3 (Jung et al. 2010). As a consequence, the p16^{INK4A} tumor-suppressor protein gets up-regulated (Jung et al. 2010). Polycomb proteins and Jmjd3 are involved in the homeostasis of the p16^{INK4A} locus, which has an important role in cellular senescence. In normally growing cells, the *INK4A/Arf* locus encoding for the tumor-suppressor proteins p16^{INK4A} and p14^{ARF} (p19^{ARF} in mice) is transcriptionally silenced by H3K27 methylation and PcG binding. In response to oncogenic stress, the *INK4A/ARF* locus loses H3K27 methylation, which leads to the expression of the p16^{INK4A} protein and, ultimately, cellular senescence. p16^{INK4A} acts by inhibiting CDK4/6 upstream of the pRB-E2F pathway, whereas p14^{ARF} inhibits the p53-specific ubiquitin ligase MDM2 and thereby increases the cellular level of p53 (Gil and Peters 2006). Jmjd3, but not UTX, gets induced by oncogenic stress (BRAF and HRAS^{V12}) approximately fourfold within 2 h and up to 12-fold during the proceeding 5 days (Agger et al. 2009). In contrast, expression of Ezh2 declined significantly following RAS activation (Agger et al. 2009; Barradas et al. 2009). Jmjd3 binds predominantly just upstream of the transcriptional start site of *INK4a*, where it leads to H3K27 demethylation and loss of the PcG proteins CBX7,

Bmi1, and SUZ12 (Barradas et al. 2009), thereby reversing Polycomb repression of the locus (Agger et al. 2009). Conversely, Jmjd3 does not bind to or increase expression of the *ARF* or *INK4b* promoters (Barradas et al. 2009). Consistent with this, the level of p53, which is stabilized by p14^{ARF}, was unchanged (Barradas et al. 2009). H3K27 methylation at the *INK4a/Arf* locus is reduced by ~50% within 3 d of oncogenic stress and is barely detectable after 7 d (Barradas et al. 2009). Due to the up-regulation of *INK4A/Arf*, Jmjd3 acts as a tumor suppressor by leading stressed cells into senescence that, at least in part, counteracts the oncogenic properties of the H3K27 HMT Ezh2. Therefore, overexpression of Jmjd3, but not UTX or loss of PcG proteins, leads to an increased expression of *INK4A* and an inhibition of cell proliferation (Jacobs et al. 1999; Agger et al. 2009; Barradas et al. 2009). Conversely, small hairpin RNA (shRNA)-mediated depletion of Jmjd3 inhibits stress-induced p16^{INK4A} and p19^{Arf} expression, which leads to immortalization of primary mouse embryonic fibroblasts (MEFs) (Agger et al. 2009). In human fibroblasts, Jmjd3 activated *Ink4A*, but not *Arf*, whereas in MEFs, Jmjd3 activates both *Ink4A* and *Arf* (Barradas et al. 2009).

Similar to Jmjd3, UTX binds predominantly upstream of transcriptional start sites of genes enriched in H3K4me2, consistent with the reported association of UTX with MLL4/ALR (Cho et al. 2007; Issaeva et al. 2007; Lan et al. 2007; Lee et al. 2007) and MLL3 (Cho et al. 2007). MLL4/ALR is necessary for UTX occupancy of some, but not all, of its target genes (Issaeva et al. 2007). Importantly, UTX is necessary and sufficient to mediate cell cycle arrest of primary human fibroblasts (Wang et al. 2010). UTX mediates cell cycle arrest by binding to and demethylating 49 genes coding for proteins that bind to the retinoblastoma (RB) tumor suppressor, which inhibits the G₁-S transition and enforces terminal differentiation (Wang et al. 2010). These proteins are coordinately down-regulated in various human cancers, including human acute myelogenous leukemia and human B-cell lymphomas (Xiang et al. 2007). Furthermore, the *RB* gene and its related gene *Rbl2* are direct targets for UTX-dependent H3K27me3 demethylation (Terashima et al. 2010). The prognostic value of UTX is linked to Ezh2 levels: In tumors with low Ezh2, UTX has a significantly larger impact on metastases and patient survival, whereas its impact is small in Ezh2-overexpressing tumors. This suggests that excess Ezh2 or lack of UTX are redundant mechanisms for misregulating H3K27me3 levels in cancer (Xiang et al. 2007).

GENOME-WIDE ANALYSIS OF H3K27 DEMETHYLASES IN HUMAN CANCERS

Jmjd3 expression levels were found to be low in various types of primary tumors (Agger et al. 2009; Barradas et al. 2009), and the inhibition of H3K27 trimethylation has a negative effect on tumor cells (Tan et al. 2007). Therefore, higher Jmjd3 protein level or activity, associated with lower H3K27me3 levels, might be a goal for cancer therapy. Sporadic inactivating somatic mutations of UTX are present in many cancers, with a prevalence of 10% in multiple myelo-

Table 1. Overview of the Role of Jmjd3 and UTX in Transcriptional Regulation

Protein	Function	References
Jmjd3	Activation of the <i>Brachyury</i> locus during ES cell differentiation, mediated by Smad2/3 following Nodal signaling.	Dahle et al. (2010)
	Activation of Pax6, Sox1, and Nestin during neural commitment in differentiating ES cells.	Burgold et al. (2008)
	Smad1/4-dependent H3K27 demethylation of the Noggin promoter following BMP signaling.	Akizu et al. (2010)
	Activation of the <i>Mash1/Ascl1</i> gene during neuronal differentiation.	Dai et al. (2010)
	Activation of genes repressed by NCoR2/SMRT during retinoic acid-induced neuronal differentiation.	Jepsen et al. (2007)
	Activation of a few hundred genes following LPS activation in mouse macrophages.	De Santa et al. (2007, 2009)
	Activation of the <i>interferon-γ</i> (<i>Ifn-γ</i>) locus following differentiation of T lymphocytes into the Th1 lineage.	Lewis et al. (2007); Miller et al. (2008)
	Activation of genes critical for epidermal differentiation.	Sen et al. (2008)
	Induction of genes involved in wound healing in murine skin repair.	Shaw and Martin (2009)
	Activation of the <i>INK4A</i> tumor-suppressor locus following oncogenic stress and senescence.	Agger et al. (2009); Barradas et al. (2009); Jung et al. (2010)
UTX	Activation of myogenin and muscle creatine kinase during myogenesis.	Seenundun et al. (2010)
	Demethylation of <i>HOXA1–3</i> and <i>HOXB1–3</i> genes during retinoic acid-induced differentiation of human pluripotent cells.	Agger et al. (2007); Lee et al. (2007)
	Regulation of <i>HOXD10–12</i> in HeLa cells (exclusive to UTX; no involvement of Jmjd3).	Lan et al. (2007)
	Activation of genes coding for proteins binding to the retinoblastoma (RB) tumor-suppressor gene.	Wang et al. (2010)
	Demethylation and activation of the <i>RB</i> gene and its related <i>Rbl2</i> gene.	Terashima et al. (2010)

ma, 8% in esophageal squamous cell carcinomas, and 1.4% in renal cell carcinomas (van Haaften et al. 2009; Dalglish et al. 2010). Induction of UTX expression in UTX null cancer cells reduced H3K27me3 and slowed cell proliferation (van Haaften et al. 2009). Down-regulation of Jmjd3 was found in lung and liver cancers (Agger et al. 2009; Barradas et al. 2009). However, despite the evidence that HDMs might act as tumor suppressors, it remains to be determined whether observed mutations are a cause or consequence of tumorigenesis. Similar to down-regulation of Jmjd3, up-regulation of the H3K27 HMT Ezh2 was found to be a prognostic marker for aggressive breast cancer and a promoter of neoplastic transformation of breast epithelial cells (Kleer et al. 2003; Cao et al. 2008). Similarly, Ezh2 is up-regulated in aggressive prostate tumors, where it transforms benign prostate epithelial cells (Varambally et al. 2002; Yu et al. 2007), bronchial cancer (Breuer et al. 2004), melanoma (Bachmann et al. 2006), bladder cancer (Weikert et al. 2005), and liver cancer (Sudo et al. 2005). Conversely, Jmjd3 is up-regulated in prostate cancers and is highest in metastatic tumors (Xiang et al. 2007).

CONCLUSIONS

It has become clear in recent years that histone methylation is not an irreversible chromatin modification and that HMTs and demethylases mediate the steady-state level of histone methylation on one hand and the plasticity for changes in gene expression on the other hand (see Table 1). However, how dynamic the methylation status of a single histone protein really is during the cell cycle, differentiation, or transcription remains to be determined. How histone demethylase activity is regulated is largely unknown and will be pivotal to our understanding of the interplay between histone methylation and demethylation. Long noncoding

RNAs have recently been shown to be a component of histone-modification complexes (Tsai et al. 2010), but whether they have an active regulatory role or merely a structural function is unknown. Furthermore, the dynamic changes of other histone modifications and how they influence histone methylation are also largely unknown. Finally, further studies will determine whether histone demethylases have additional role(s) in transcriptional regulation that are independent of their catalytic activity or whether they regulate other nonhistone substrates.

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M.R. Hübner and D.L. Spector

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