Role of H3K27 Demethylases Jmjd3 and UTX in Transcriptional Regulation

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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 sole 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). In embryonic stem (ES) cells, 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 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; Mosammeparast 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 Whetstine 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.
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. 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 (H4K4me3) 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 JmjdC 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 demethylylation 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. 2006; Mikkelsen et al. 2007; Pan et al. 2007; Zhao et al. 2007).
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-xB to conserved NF-xB-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 Ifn-γ genes are Jmjd3 targets in dif-ferentiating 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 with MLL1, as well as with the MLL complex components Wdr5, RbBP5, and Ash2L. 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 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).

RA-induced differentiation of human pluripotent embryonal 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 XI93 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 MLL2/3, which leads to demethylation of H3K27me2/3, H3K4 methylation, and the loss of PRC1-mediated transcriptional repression (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). How-
ever, the depletion of UTX and the concomitant increase in H3K27 methylation did not have a significant effect on the occupancy of the HOX113 and HOX14 promoters with components of the MLL2/3 complex or 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 knockdown (Lan et al. 2007). Additionally, the 3′ end of HOXD12 had a higher H3K27me3 level in UTX-depleted cells. In contrast, Jmd3 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 exclusively 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 SENESCENCE

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 Jmd3 (Jung et al. 2010). As a consequence, the p16INK4A tumor-suppressor protein gets up-regulated (Jung et al. 2010). Polycomb proteins and Jmd3 are involved in the homeostasis of the p16INK4A locus, which has an important role in cellular senescence. In normally growing cells, the INK4A/Arf locus encoding for the tumor-suppressor proteins p16INK4A and p14ARF (p19ARF in mice) is transcriptionally silenced 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 HUMAN CANCERS

Jmd3 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 Jmd3 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 myeloma, 8% in esophageal squamous cell carcinomas, and 1.4% in renal cell carcinomas (van Haaf et al. 2009; Dalglish et al. 2010). Expression of UTX expression in UTX-mutated cancer cells reduced H3K27me3 and slowed cell proliferation (van Haaf et al. 2009). Down-regulation of Jmd3 was found in lung and liver cancers (Agger et al. 2009; Barradas et al. 2009). However, despite the evidence
Table 1. Overview of the Role of Jmjd3 and UTX in Transcriptional Regulation

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