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Epigenetic Modifications in Distinction: Histone versus DNA Methylation in ESCs

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In this issue of *Cell Stem Cell*, Karimi et al. (2011) show that DNA methylation and histone H3 lysine 9 trimethylation (H3K9me3) have distinct genomic targets in mouse ESCs. In particular, loss of H3K9me3 leads to derepression of select endogenous retroviruses and subsequent ectopic transcription of adjacent genes.

Epigenetic mechanisms, including DNA methylation, histone tail modifications, and noncoding RNAs (nc-RNAs) such as microRNA, lincRNAs, and piRNAs, can stably influence gene expression without changing the underlying DNA sequence. While each type of epigenetic mechanism can exert a unique influence on a subset of genes, they collectively act in concert with core transcriptional circuitry to compose the transcriptome landscape in different cell types (Fouse et al., 2008). Alteration of one epigenetic factor may also lead to changes in another epigenetic pathway. For example, both DNA methylation and repressive histone modifications (including H3 deacetylation and lysine 9 methylation) are associated with repressive chromatin remodeling in gene silencing (Cedar and Bergman, 2009). Loss of histone H3K9me2 modification in the absence of lysine methyltransferase G9a can cause a significant reduction of DNA methylation in multiple genomic regions, including promoters, satellite repeats, and retrotransposons (Dong et al., 2008).

Genetic studies indicate that DNA and histone methylation are essential for animal development; mutant mice lacking either DNA methyltransferase (DNMT) or histone lysine methyltransferases (KMTase) exhibit a lethal phenotype. However, DNMTs and KMTases are not essential for self-renewal of pluripotent mouse embryonic stem cells (mESCs): either single Dnmt mutant ESCs (*Dnmt1*$^{-/-}$, *Dnmt3a*$^{-/-}$, *Dnmt3b*$^{-/-}$) or triple *Dnmt1/3a/3b* knockout (TKO) mESCs can proliferate and self-renew normally (Hutnick et al., 2010; Tsumura et al., 2006). Similarly, mESCs deficient for lysine 9 KMTase (SUV39H1, SUV39H2, G9a, GLP, and SETDB1) can survive (Dong et al., 2008; Matsui et al., 2010; Rowe et al., 2010). The availability of mESCs that exhibit deficits in DNA methylation and histone modifications provides a unique opportunity to understand how multiple layers of epigenetic factors are involved in regulating gene expression in pluripotent ESCs.

In this issue of *Cell Stem Cell*, Lorincz and colleagues focus on the shared and distinct functions between DNA methylation and H3K9me3, reporting a comprehensive comparison between the two epigenetic mechanisms in mouse ESCs (Karimi et al., 2011). The authors performed mRNA-Seq and ChIP-Seq of H3K9me3 in wild-type, SETDB1 KO, and DNMT TKO mESCs with the goal to assess the effect of DNA methylation and H3K9me3 on mRNA transcriptome. Consistent with previous findings (Matsui et al., 2010), the authors found that both epigenetic pathways appear to be independent from each other. For example, at SETDB1-bound regions, DNMT TKO cells show minor loss of H3K9me3 enrichment, whereas nearly 80% of H3K9me3 sites are lost in SETDB1 KO mESCs compared with control. In the reverse comparison, the authors found that proximal promoter regions bound by SETDB1 are frequently unmethylated. However, the DNA methylation data set used in this study only covers an ~4 kb region surrounding annotated proximal gene promoters (Myant et al., 2011); therefore, the overlap of DNA methylation and H3K9me3 at many genomic regions, including gene body, repeat elements, and other interesting intergenic regions, remain to be investigated.

Compared with control, derepressed genes in DNMT TKO and SETDB1 KO have little overlap, likely reflecting their distinct genomic localization. However, there is a small subset of genes that is derepressed in both KO cell lines, genes predominantly involved in germline development. Thus, both DNA methylation and H3K9me3 are required for the silencing of these germline-related genes. While the authors demonstrate reduced levels of H3K9me3 in DNMT TKO cells at these genes, it would be of interest to determine whether these loci still retain or lose DNA methylation in SETDB1 KO cells. Nevertheless, derepression of this small subset of genes in both DNMT TKO and SETDB1 KO cells indicates that DNA methylation and H3K9me3 may be involved in the same regulatory pathway.

Previous studies have shown that H3K9me3, established by SETDB1, is important for silencing subfamilies of endogenous retroviruses (ERVs), a class of repeat element found throughout the mammalian genome (Matsui et al., 2010; Rowe et al., 2010; Hutnick et al., 2010). ERVs are strictly inactivated during embryogenesis to prevent insertional mutations during early development (Rowe and Trono, 2011). With the powerful technique of RNA-Seq, the authors now identified an entire slew of ERV subfamilies, predominantly in class I and class II, derepressed exclusively in SETDB1 KO cell lines. The proximal regions flanking ERV elements showed consistent and marked loss of H3K9me3 in SETDB1 KO, indicating a direct and genome-wide role for SETDB1 regulating ERVs. In contrast, loss of DNA methylation in DNMT TKO cells had minimal effects on ERV expression. Interestingly, double *Dnmt1/Setdb1* knockdown in wild-type mESCs showed synergistic effects only at IAPE-z repeat element, suggesting DNA methylation plays a minor role in silencing select retroviral elements in mESCs.

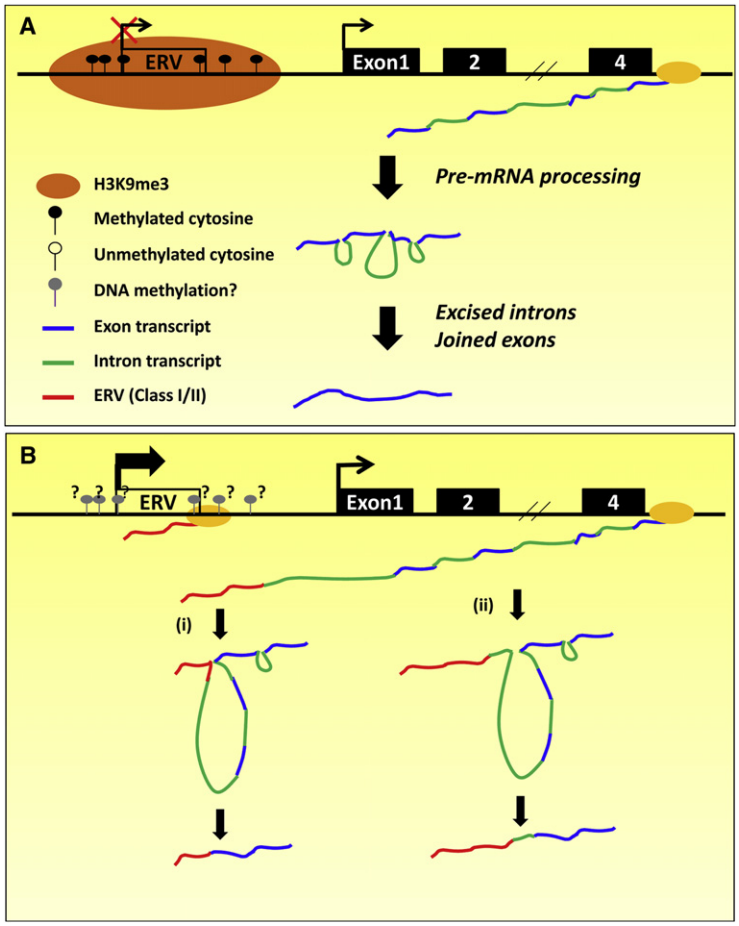

Figure 1. ERV Expression Promotes Ectopic Transcription of Adjacent Genes
Schematic illustrating the effects of H3K9me3 on ERV expression. (A) ERVs are silenced by H3K9me3, and normal transcription occurs in wild-type mESCs. (B) Loss of H3K9me3 in SETDB1 KO mESCs triggers ectopic activation of ERVs, promotes increased expression of adjacent genes, and generates chimeric transcripts. (i) and (ii) are typical alternative 3′ donor splice sites either in the ERV body (i) or in the 3′ flanking genomic region (ii).

One of the most interesting findings of this paper is that in SETDB1 KO mESCs, ectopic activation of ERVs also triggers transcription of nearby genes, especially if the ERV element was within 5 kb upstream of the transcription start site (TSS). Notably, the authors were able to identify "chimeric" transcripts—pair-end reads mapping to both an ERV element and a genic exon—associated with ectopic transcript activation. Genes with chimeric transcripts are typically not enriched in H3K9me3, unlike the upstream ERV element. Furthermore, genes with chimeric transcripts ranked among the highest expressed genes, indicating a role for ERV in promoting adjacent protein-coding RNA expression (Figure 1).

This study provides an excellent example of unraveling the many layers of epigenetic mechanisms in silencing retroviral elements in mESCs by using SETDB1 KO and DNMT TKO cells. Many interesting questions remain to be resolved with regards to the specific actions of each major epigenetic regulator in maintaining genome stability in mammalian cells. For example, would ectopic activation of ERVs in SETDB1 KO ESCs cause an increase in retrotransposon insertions and overall genomic instability? Are there any specific changes in small RNAs or nc-RNA transcriptome in DNMT TKO or SETDB1 KO ESCs? Do H3K9me3 and DNA methylation play similar roles in gene silencing of ERVs in differentiated somatic cells? With the increased analytical powers of high-throughput sequencing, we look forward to seeing more exciting results in the near future.

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