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Adaption by Rewiring Epigenetic Landscapes

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Abstract

Embryonic stem cells (ESCs) generally rely on repressive histone modifications to silence retrotransposons, rather than DNA methylation as in differentiated cells. In this issue of *Cell Stem Cell*, He et al. (2015) show that Daxx/Atrx repress transposons in ESCs devoid of 5mC, demonstrating dynamic reorganization of epigenetic networks and crosstalk between distinct repressive mechanisms to maintain transposon silencing.

In his 1957 classic (Waddington, 1957), "The Strategy of Genes," Conrad Waddington proposed that cells undergo Darwinian adaptions at the molecular level in response to environmental changes and/or mutations. Such adaptions would be beneficial for the stabilization of a species, allowing genomes to resist constant molecular fluctuations (such as alteration of gene expression or emergence of new genes) during evolution. He also proposed that epigenetic regulation, which he defined in much broader terms than simply regulation of chromatin structure, must be a critical component of such adaptions because it doesn't require change of the genetic material and therefore represents a mechanism for rapid response to such fluctuations. Five decades later, we have only begun to appreciate the significance of this idea, although "epigenetic landscape," a term coined in the same book, has gained much popularity. In this issue of *Cell Stem Cell*, Songyang and colleagues (He et al., 2015) demonstrate a perfect example of such adaptions by revealing an intricate reorganization of epigenetic networks to silence retrotransposons in embryonic stem cells (ESCs).

Transposable elements (TEs) are remnants of ancient viruses that invaded host genomes and later became domesticated. According to their mechanisms of transposition (Wicker et al., 2007), TEs are generally divided into two major classes, retrotransposons and DNA transposons (Figure 1A). Retrotransposons, which are the focus of the work presented herein (He et al., 2015), must be transcribed to RNA and then reverse transcribed to DNA in order to reintegrate into the host genome in a process called retrotransposition. Therefore, transcription of retrotransposons is kept under strict control to prevent insertional mutagenesis. Although the majority of retrotransposons have already been fossilized during mammalian evolution, several sub-family members, such as LINE1, IAPs, and MuERV-L, remain active during particular stages of embryogenesis. On one hand, retrotransposons can influence gene expression by providing alternative promoters, splicing donor sequences, or

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polyadenylation signals. Recent studies have shown that retrotransposon derepression re-wires host cell transcription networks and plays critical roles in embryogenesis (Peaston et al., 2004) and regulating the self-renewal of pluripotent stem cells (Wang et al., 2014). On the other hand, retrotransposition may pose a significant threat to genome stability. For instance, it was estimated that approximately 10% of de novo mouse germline mutations are caused by IAP remobilizations (Maksakova et al., 2006).

Retrotransposon transcription is controlled by two epigenetic pathways: DNA methylation (5 methyl-Cytosine, 5mC) and repressive histone modifications (mainly H3K9me2/3), which, in principle, repress transposons in differentiated cells and ESCs, respectively. For example, IAPs are only detectable in the differentiating *Dnmt1*$^{-/-}$ ESCs (Hutnick et al., 2010), which is consistent with the in vivo finding that IAPs are strongly upregulated in *Dnmt1*$^{-/-}$ postimplantation mouse embryos. However, in an established ESC line that lacks all DNA methyltransferases (*Dnmt1/Dnmt3a/Dnmt3b* triple knockout, TKO), retrotransposons lose nearly all 5mC but still remain largely silenced (Arand et al., 2012).

Herein, Songyang and colleagues (He et al., 2015) revealed an unexpected connection between these mechanisms in ESCs. Daxx and Atrx are known to play critical roles in transcriptional control and epigenetic regulation, and the authors now find that these proteins also work to silence repetitive sequences, including a subset of retrotransposons and TERRA (subtelomere RNA transcripts), in response to a decrease in 5mC levels (Figure 1B). First, they observed from ChIP-seq experiments that a number of Daxx and Atrx peaks are localized at retrotransposon sequences in wild-type (WT) cells. Surprisingly, they found that both Daxx and Atrx gain significantly more peaks in TKO ESCs, and these peaks are localized to LTR retrotransposons and other repetitive sequences. These results strongly suggest that Daxx and Atrx have significant roles in transposon silencing, especially in 5mC-deficient cells.

To validate this hypothesis, the authors completed a series of mRNA-seq experiments to show that a subset of LTR retrotransposons (IAPs, ETn/MusD, MMERVK, and RLTR4) is indeed activated when Daxx is deficient. In TKO ESCs or 2i-cultured ESCs in which DNA is hypomethylated, Daxx or Atrx deficiency dramatically activates the aforementioned retrotransposons and results in severe telomere abnormalities. They next set out to understand how Daxx and Atrx silence TEs in the absence of 5mC. The authors performed immunofluorescence staining experiments and found that Daxx and Atrx are redistributed from pericentric heterochromatin to telomeres in TKO cells, which coincides with increased H3K9me3/HP1a deposition. Increased H3K9me3 levels were also found at other Daxx binding sites, including IAPs and subtelomeres. Then they demonstrated that redistribution of Daxx/Atrx complex to telomeres in TKO ESCs is dependent on Daxx, but not Atrx. In addition, rescuing 5mC levels by overexpression of either Dnmt3a or Dnmt3b, but not their catalytically dead mutants, restored Daxx/Atrx localization at pericentric heterochromatin. These results strongly suggest that Daxx recruitment is inversely correlated with 5mC deposition. Furthermore, the authors showed that Daxx interacts with H3K9me3 methyltransferase Suv39h1, which suggests that Suv39h1 is responsible for the increased H3K9me3 levels at transposons and telomeres/subtelomeres. A key question, however, that remains unanswered is how Daxx/Atrx/Suv39h1 are redeposited at transposons and

telomeres, which mostly likely involves factors and pathways that can sense altered 5mC levels in TKO cells.

The authors extended their investigation to early embryogenesis. They showed that in cleavage embryos that undergo rapid 5mC demethylation, depletion of Atrx leads to elevated IAP expression. Furthermore, as 5mC levels progressively decrease in embryos advancing from the zygote stage to morula stage, deposition of Daxx and Atrx increases at telomeres. Taken together, this study shows how two distinct epigenetic pathways cooperatively repress retrotransposons and protect genome integrity in mouse ESCs. It would be of great interest to know whether Daxx has a similar function in human cells.

In summary, this study sheds new light on epigenetic adaption mechanisms in ESCs. Mammalian cells have evolved various sophisticated defense mechanisms against genomic parasites, and it is conceivable that more mechanisms await discovery. Furthermore, this study also raises many intriguing questions: What are the mechanisms for *Daxx* and *Atrx* redistribution in the DNA hypomethylated ESCs and cleavage embryos? Why are *Dnmt1*-deficient embryos and differentiating ESCs unable to adapt to lower levels of 5mC and silence transposons? Finally, do pluripotency genes play direct roles in the epigenetic silencing of retrotransposons? The answers to these questions will help us understand how cells adapt to genomic fluctuation, and they may be essential to further understand pluripotency.

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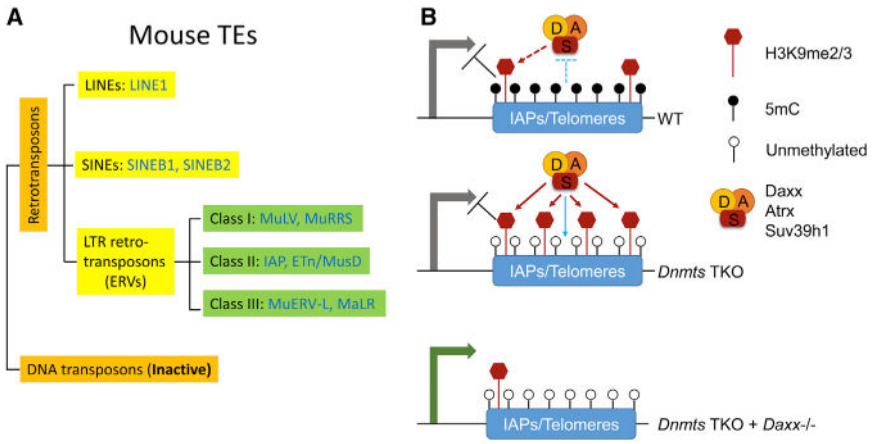

Figure 1. DAXX-Centered Epigenetic Repression

(A) An abbreviated classification of transposable elements (TEs) in mice. Active members are shown in blue. LINE, long interspersed element; SINE, short interspersed element; ERV, endogenous retrovirus; MuLV, Murine Leukemia Virus; MuRRS, murine retrovirus-related sequence; IAP, intracisternal A-particle; ETn, early transposon; MuERV-L, murine endogenous retrovirus-L; MaLR, mammalian long terminal repeat transposon.

(B) Wild-type ESCs (upper panel) use repressive histone marks of H3K9me2/3 and 5mC to silence TE and telomeres, in which the Daxx/Atrx/Suv39h1 binding seems attenuated. In TKO ESCs that lack 5mC (middle panel), enhanced Daxx/Atrx/Suv39h1 occupancy at retrotransposons and telomeres results in increased H3K9me3 levels and maintenance of TE silencing. Simultaneous loss of 5mC and Daxx (lower panel) leads to TE derepression and telomere dysfunction. Transcription levels are indicated by the color-coded arrows; a gray arrow signifies transcription inactivation and a green arrow signifies transcription activation.