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Retrovirus Silencing by an Epigenetic TRIM

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DOI 10.1016/j.cell.2007.09.029

Embryonic cells silence transcription by retroviruses, but how do they recognize virus DNA? In this issue, Wolf and Goff (2007) report that a TRIM28 corepressor complex binds to the retrovirus primer binding site. Epigenetic silencing of retrovirus transcription is accomplished by "writing" a dimethyl mark on lysine 9 of histone H3 that is read by the heterochromatin protein HP1γ.

The mammalian genome is defended in the early embryo by restricting the expression of foreign DNA elements. It has been known for 30 years that embryonic cells in the mouse silence retrovirus transcription via epigenetic mechanisms. Retrovirus silencing may be a common feature of stem cells in contrast to differentiated cells that express retrovirus efficiently. Despite many efforts to delineate the pathways involved, the silencing process remains poorly understood. One elusive aspect of retrovirus silencing depends upon recognition of the viral Pro^{tRNA} primer binding site (PBS) by a cellular factor that has been notoriously difficult to purify. The PBS confers a profound silencing effect on retrovirus vectors in embryonic stem cells and embryonic carcinoma cells, which is relieved by a single point mutation known as B2 (Barklis et al., 1986). In this issue of *Cell*, Wolf and Goff (2007) identify a TRIM28 corepressor complex that binds to the PBS in embryonic cells revealing the importance of this complex in retrovirus silencing. Furthermore, their results implicate histone H3 lysine 9 (H3K9) dimethylation and HP1γ in the epigenetic silencing of retroviruses in vivo.

Wolf and Goff isolated the PBS-binding complex from embryonic carcinoma cells by sequential chromatography and examined fractions that bound to PBS but not B2 probes. Mass spectrometry of the complex bound to the PBS identified the TRIM28 corepressor. Inducing embryonic carcinoma cells to differentiate with retinoic acid resulted in the disappearance of both TRIM28 and the PBS-binding complex as expected. Knockdown of TRIM28 in embryonic stem cells and embryonic carcinoma cells using RNA interference (RNAi) reduced the amount of protein bound to the PBS that was rescued by overexpression of an RNAi-resistant TRIM28. These findings demonstrate that TRIM28 is essential for PBS binding in vitro, but what does it do in vivo?

TRIM28 is a tripartite motif protein 28 (Kap-1/Tif1-beta) that is a universal corepressor recruited to DNA by many different Kruppel-associated box zinc finger (KRAB-ZNF) proteins (Urrutia,

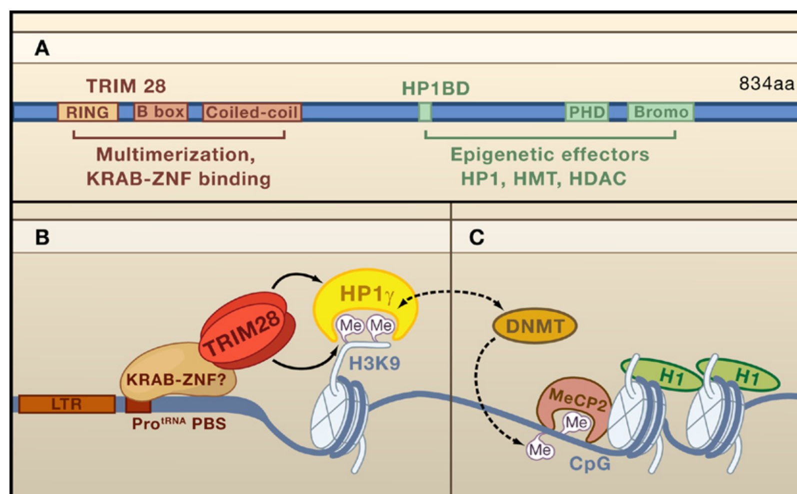

Figure 1. Retrovirus Silencing by a TRIM28-Mediated Epigenetic Cascade
TRIM28 acts as a corepressor by tethering epigenetic effectors to the target DNA site, resulting in a condensed nucleosome structure that silences transcription.
(A) TRIM28 protein motifs mediate subunit multimerization and interaction with a KRAB-ZNF protein that recognizes target sites. Other motifs tether epigenetic effectors to the complex including histone methyltransferase (HMT), histone deacetylase (HDAC), and HP1 family members.
(B) Wolf and Goff (2007) demonstrate that the retrovirus primer binding site (PBS) adjacent to the 5′ long terminal repeat (LTR) is bound in vivo by a large TRIM28 corepressor complex that triggers the writing of nucleosomal histone H3K9 dimethylation marks that are read by HP1γ. The DNA-binding subunit may be a KRAB-ZNF protein. A histone deacetylase may ensure that H3K9 is deacetylated and available for a histone methyltransferase that writes the dimethyl mark.
(C) Companion silencing marks may be recruited in self-sustaining epigenetic cascades that ensure the retrovirus is not reactivated in embryonic cells. Dotted arrows indicate possible HP1γ interactions with DNA methyltransferase (DNMT) and subsequent CpG methylation marks that are bound by MeCP2. Histone H1, also found in retroviruses that are transcriptionally silenced, promotes chromatin condensation.

2003). The KRAB-ZNF proteins have multiple DNA-binding domains and therefore are capable of recognizing large DNA motifs. Once targeted to DNA by a KRAB-ZNF protein, TRIM28 initiates epigenetic silencing by recruiting histone deacetylase, histone methyltransferase, and HP1 family members (Figure 1A). Other TRIM proteins have properties that restrict HIV-1 and other viruses, but these act via different mechanisms.

Wolf and Goff took two approaches to determine the potential function of TRIM28 in retrovirus silencing in vivo. First, they infected embryonic carcinoma cells with murine leukemia virus vectors to show that PBS-mediated silencing is abrogated in TRIM28 knockdown cells. Second, they performed chromatin immunoprecipitations on a pair of cell lines bearing a retrovirus integrated at the same site. The retrovirus with a PBS site is silent, bound by TRIM28, and marked by H3K9 dimethylation and HP1γ (Figure 1B). The retrovirus with a B2 site is active and not bound by TRIM28 or silent chromatin marks. These results confirm that the TRIM28 corepressor binds to the PBS in vivo and directs retrovirus silencing by recruiting marks of silent chromatin.

Many fascinating questions are stimulated by these new findings. What confers the specificity that silences retrovirus transcription in embryonic cells? TRIM28 is widely expressed in many cell types that do not silence retrovirus vectors, suggesting that other subunits of the complex may define this embryonic/stem cell-specific capability. Wolf and Goff note that TRIM28 has been reported to interact with the pluripotent stem cell marker Nanog. It is conceivable that TRIM28 regulates, interacts, or cooperates with the pluripotency factors used by Takahashi and Yamanaka (2006) to induce pluripotent stem (iPS) cell formation from fibroblasts. Once fibroblasts are reprogrammed into iPS cells, they silence retrovirus vectors (Okita et al., 2007). Thus, pluripotency factors or their transcriptional networks may directly or indirectly control retrovirus silencing.

How is TRIM28 targeted to the PBS? Wolf and Goff speculate that several TRIM28 subunits are present in the PBS complex and that DNA recognition is governed by a KRAB-ZNF protein (Figure 1B) that they were unable to detect. A candidate approach to this issue is hampered by the presence of 290 KRAB-ZNF proteins in the mouse. Indeed, the cell specificity of retrovirus silencing might be simply explained if a PBS-specific KRAB-ZNF protein were to be expressed only in embryonic cells. Synthetic KRAB-ZNF proteins that target the PBS have already been used to silence HIV-1 expression and have therapeutic potential.

How can the tantalizing epigenetic data be extended toward fully unraveling the silencing mechanism? Reconstruction of the complete epigenetic pathway will require extensive chromatin immunoprecipitation and functional knockdown studies. It is critically important to determine the histone methyltransferase responsible for writing the H3K9 dimethylation mark read by HP1γ (Figure 1B). HP1γ also regulates HIV-1 latency but in this context is targeted to H3K9 trimethylation marks written by Suv39H1 (du Chene et al., 2007). HP1γ interacts with DNA methyltransferases (Brenner and Fuks, 2007) and therefore may facilitate de novo DNA methylation of silent retrovirus and loading of the protein MeCP2 that binds to methylated DNA (Lorincz et al., 2001). Additional marks in silenced retrovirus and lentivirus include deacetylated histone H3 and the presence of histone H1 (Yao et al., 2004), implying the existence of additional companion pathways (Figure 1C). Hence, many layers of epigenetic marks maintain the silent state to prevent retrovirus reactivation. Self-sustaining epigenetic feedback loops may reinforce the system by mutual recruitment of histone methyltransferases, HP1s, DNA methyltransferases, histone deacetylases, and histone H1.

Will identification of TRIM28 facilitate genetic manipulation of stem cells with retrovirus vectors? Retrovirus vectors have been generated with the B2 mutation or a Gln^{tRNA} site that escape PBS-mediated silencing. However, these optimized vectors are still frequently silent or exhibit low expression in stem cells and are subject to position effects that can lead to variegated expression that is modulated by transcriptional noise (Ramunas et al., 2007; Swindle et al., 2004). A better understanding of the PBS-mediated silencing pathway will be instructive for understanding the companion mechanisms and thereby contribute to the future design of retrovirus vectors that will potently and reproducibly express transgenes in stem cells.

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