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Revealing the Complexity of Retroviral Repression

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Retroviral restriction is a complex phenomenon that, despite remarkable recent progress, is far from being well understood. In this Preview, we introduce an insightful study by Yang et al. that represents the first attempt to identify the global determinants of retroviral repression in pluripotent mammalian cells.

To protect their genomic integrity, animals control retroviral infections by establishing heritable epigenetic silencing of the integrated provirus in early embryonic development. In mouse embryonic stem cells (ESCs), KAP1 (Trim28) is targeted to newly integrated Moloney murine leukemia virus (MMLV) by the Krueppel-associated box (KRAB) zinc finger protein ZFP809. KAP1, in turn, recruits histone-modifying enzymes, including the histone methyl transferase SETDB1 (ESET), that deposit repressive histone 3 lysine 9 trimethylation (H3K9me3) marks at the provirus (Figure 1) (Matsui et al., 2010; Rowe et al., 2010; Wolf and Goff, 2009). The KRAB/KAP1 system also represses endogenous retroviruses (ERVs), which are potentially hazardous remnants of retroviral germline infections (Matsui et al., 2010; Rowe et al., 2010; Wolf et al., 2015). Additionally, several cofactors of the KRAB/KAP1 system, but also KAP1-independent retroviral repression pathways, have been identified over the last few years. Indeed, the abundance and sequence diversity of exogenous and endogenous retroviruses likely drove evolution of complex and partially redundant repression mechanisms that keep these elements under control. Moreover, some ERVs have been adapted as new regulatory elements and, in some cases, have re-wired entire transcriptional networks (Macfarlan et al., 2012). Retroviral repression mechanisms might therefore also regulate transcription of cellular genes. Despite recent progress in the field, deciphering the complexity and interconnectivity of retroviral repression pathways and networks remains an outstanding problem of mammalian genome biology. The Resource article by Yang et al. (2015) performs a genome-wide small interfering RNA (siRNA) knockdown screen in a first attempt to determine in a global manner the components of retroviral repression machinery in mammalian pluripotent cells.

The siRNA screen was performed using a MMLV reporter that is repressed by ZFP809/KAP1 and is therefore primarily aimed at identifying cofactors acting up- and downstream of the KRAB/KAP1 system, but also at potentially overlapping KAP1-independent repression pathways. Apart from previously known factors, including ZFP809, KAP1, and SETDB1, hundreds of new repression candidates were identified. As expected, many candidates are associated with chromatin modification, DNA methylation, and regulation of transcription. Additionally, the screen identified genes involved in protein sumoylation, DNA repair, and DNA replication and even factors located outside of the nucleus (e.g., plasma membrane, cytoskeletal, and organelle proteins). These findings highlight the complexity of retroviral restriction networks in mammalian cells, although many of these factors may not primarily, specifically, and/or directly repress retroviruses. Without a doubt, the provided candidate list is a potentially valuable resource for future studies that may address how these factors mediate retroviral restriction and ultimately help us to better understand how epigenetic silencing of retroviruses is established, maintained, and inherited during development.

Two of the newly identified repression mechanisms are subsequently analyzed

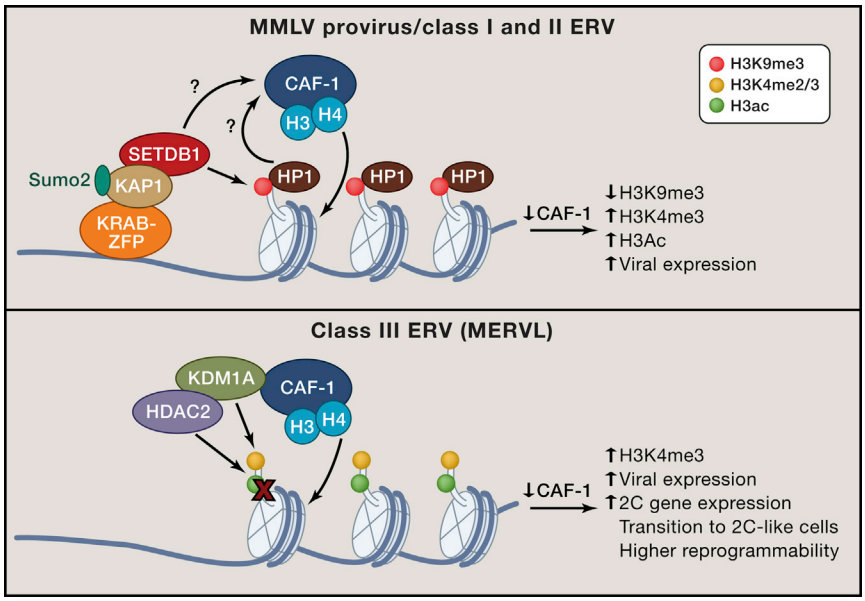

Figure 1. SUMO2/CAF-1-Assisted Retroviral Silencing

Simplified models of SUMO2/CAF-1-assisted retroviral silencing are depicted. (Top) The MMLV provirus and some endogenous retroviruses are targeted by various KRAB zinc finger proteins (KRAB-ZFPs) that recruit the KAP1 corepressor. KAP1, among other functions, recruits SETDB1, which deposits the repressive H3K9me3 mark at histone H3. HP1, a reader of the H3K9me3 mark, may recruit CAF-1. Alternatively, SETDB1, which immunoprecipitates with CAF-1, might be involved in CAF-1 recruitment to the proviral DNA. In concert with other factors, CAF-1 delivers a H3-H4 dimer onto the retroviral DNA during replication to maintain the repressive mark. (Bottom) MERVL elements are not repressed by the KRAB/KAP1/SETDB1 complex and lack the H3K9me3 mark. Instead, MERVL ERVs are repressed by the H3K4 lysine demethylase KDM1A and histone deacetylases, such as HDAC2, which both remove histone modifications associated with open chromatin and transcription. CAF-1 knockdown results in epigenetic changes and retroviral de-repression, but also to the activation of MERVL-regulated 2C genes. This ultimately facilitates the transition of ESCs to a more epigenetically pliable state, similar to 2C embryos.

in detail to validate the significance of the screen: KAP1 sumoylation and chromatin assembly at proviruses. Yang et al. show that KAP1 sumoylation by SUMO2 is required for KAP1 recruitment to the MMLV provirus and ERVs and thus for epigenetic silencing of these elements. This supports previous findings that sumoylation of KAP1 by SUMO2 is essential for forming a stable KRAB/KAP1/SETDB1 repression complex (Ivanov et al., 2007). CHAF1A, one of the top hits in the screen, is the core component of the chromatin assembly factor-1 (CAF-1). CAF-1 depletion impairs repression of newly integrated proviruses and also promotes reactivation of several ERV families, many of which are bound by both CAF-1 and KAP1 (Yang et al., 2015). Although this implies that CAF-1 is a component of the KRAB/KAP1 silencing system, SUMO2 knockdown, which results in KAP1 loss at the MMLV provirus, does not disrupt CAF-1 binding (Yang et al., 2015). This indirectly indicates that CAF-1 recruitment to retroviruses is independent of KAP1 binding. The question remains: how is CAF-1 targeted to retroviral elements? One possibility is that CAF-1 re-assembles histones at repressed retroviral elements after DNA replication and thus aids to maintain heterochromatin marks, as previously suggested (Yu et al., 2015). In this model, free histone H3, mono-methylated by SETDB1, is incorporated into newly synthesized heterochromatic DNA by CAF-1 and is further methylated to form stable heterochromatin on the newly synthesized strand via heterochromatin protein 1 (HP1), which binds to the H3K9me3 mark (Yu et al., 2015). CAF-1 immunoprecipitates with both SETDB1 and HP1, possibly explaining its localization at retroviral elements (Figure 1) (Yang et al., 2015; Yu et al., 2015). However, it has yet to be determined whether CAF-1 localization at KAP1-controlled ERVs is indeed replication dependent and whether the chromatin assembly function and/or the PCNA-HP1 interacting function of CAF-1 is required for ERV silencing. Furthermore, it remains open whether CAF-1 localizes exclusively at ERV-associated heterochromatin or also at non-viral genes that are repressed by KRAB/KAP1, for example, at imprinted genes. Nevertheless, the findings provided by Yang et al. strongly support a role for CAF-1 in the establishment and/or maintenance of heterochromatin at ERVs after DNA replication.

Interestingly, CAF-1 is also recruited to ERVs that are not bound by KAP1, SETDB1, or H3K9me3—especially class III ERVs, which consist primarily of MERVL elements (Figure 1). These elements are among the ERVs with the highest reactivation levels in CAF-1-depleted cells (Yang et al., 2015), an observation supported by a recent report (Ishiuchi et al., 2015). This indicates that CAF-1 may target and repress different ERV classes by entirely different mechanisms. Previously, it has been shown that MERVL repression by CAF-1 requires the chromatin-assembly activity of CAF-1, but not its functional interaction with HP1 and PCNA (Ishiuchi et al., 2015). Moreover, growth arrest of CAF-1 knockdown ESCs at G1-S prevented MERVL reactivation, indicating that CAF-1 acts to repress MERVL elements during or after DNA replication (Ishiuchi et al., 2015). Yang et al. also show that KDM1A (LSD1), which physically interacts with CAF-1, is strongly enriched at CAF-1-bound MERVL elements (Yang et al., 2015). KDM1A represses MERVL elements in ESCs (Macfarlan et al., 2011), but KDM1A binding at MERVL ERVs has not been previously demonstrated. However, it remains open how CAF-1 and KDM1A are targeted to these elements.

Depletion of KDM1A in ESCs leads to de-repression of MERVL transcripts and MERVL-associated genes, but also to an increased number of spontaneously arising cells resembling two-cell-stage embryos (2C-like cells) within the ESC population (Macfarlan et al., 2012). Interestingly, CAF-1 knockdown ESCs exhibited similar phenotypes (Ishiuchi et al., 2015). Moreover, the nuclei of 2C-like cells originating from CAF-1 knockdown ESCs are also

shown to lack chromocenters and are more efficiently reprogrammed by nuclear transfer into enucleated oocytes (Ishiuchi et al., 2015), supporting the important link between CAF-1/KDM1A-mediated retroviral repression and cellular epigenetic potential in early development.

Altogether, Yang et al. provide a valuable source for retroviral repression candidates using a genome-wide siRNA knockdown screen. Importantly, several of the newly identified factors are confirmed to function in pathways that have not been directly associated with retroviral repression before. This validation strongly supports that the screen identified bona fide candidates, whose further investigation will not only deepen our understanding of the complex retroviral restriction networks, but also reveal new regulatory mechanisms in retrovirus-derived transcriptional networks.

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