VIRAL CELL BIOLOGY

Stop HUSHing on SIV/HIV

Primate immunodeficiency virus accessory proteins Vpx/Vpr associate with and induce proteasomal degradation of the HUSH complex, thereby counteracting HUSH-mediated epigenetic transcriptional repression of proviral and cellular genes. These findings open new therapeutic avenues against HIV.

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The growth of primate immunodeficiency lentiviruses is naturally constrained by a variety of cellular restriction factors that inhibit retroviral infection at different steps in the virus replication cycle. However, human and simian immunodeficiency viruses (HIV and SIV, respectively) encode several dedicated accessory proteins (including viral infectivity factor (Vif), viral protein U (Vpu) and viral protein X/viral protein R (Vpx/Vpr)) that enable them to evade these host immune defences, thus ensuring viral survival and propagation. Vpr is encoded by all HIVs and SIVs, whereas the accessory Vpx is encoded by HIV-2 and additional SIV lineages. Vpx/Vpr proteins interact with the DCAF1 ubiquitin ligase adaptor and hijack the DCAF1–Cul4A/B E3 ubiquitin ligase complex to promote ubiquitination and proteasome-dependent degradation of cellular proteins[1]. Many of the Vpx/Vpr orthologues — but not HIV-1 Vpr — promote degradation of the cellular restriction factor SAMHD1 (sterile alpha motif and histidine/aspartic acid domain-containing protein 1), which suppresses viral reverse transcription by depleting the intracellular pool of dNTPs[2,3]. In a recent issue and the current issue of *Nature Microbiology*, respectively, Chougui et al.[4] and Yurkovetskiy et al.[5] now identify the human silencing hub (HUSH) epigenetic repressor complex as a novel degradation target of SIV/HIV accessory proteins Vpx and Vpr (but notably, not HIV-1 Vpr). These findings link intrinsic immunity and epigenetic control of gene transcription for the first time, and could lead to new antiviral therapeutic approaches.

The HUSH complex is composed of three subunits, TASOR, MPP8 and periphilin[6] (Fig. 1), and is involved in position-effect variegation, whereby the local chromatin environment at different sites of transgene integration leads to heterogeneous transgene expression levels. Through the chromodomain of MPP8, HUSH preferentially localizes to genomic loci that are rich in the repressive histone mark, tri-methylated lysine 9 of histone H3 (H3K9me3). HUSH-mediated recruitment of the histone methyltransferase SETDB1 to these sites results in the 'writing' of additional H3K9me3 (ref. [6]) (Fig. 1). Thus, the HUSH complex acts by spreading H3K9me3-modified heterochromatin, although it is also capable of repressing transcription within euchromatin[7]. In addition to

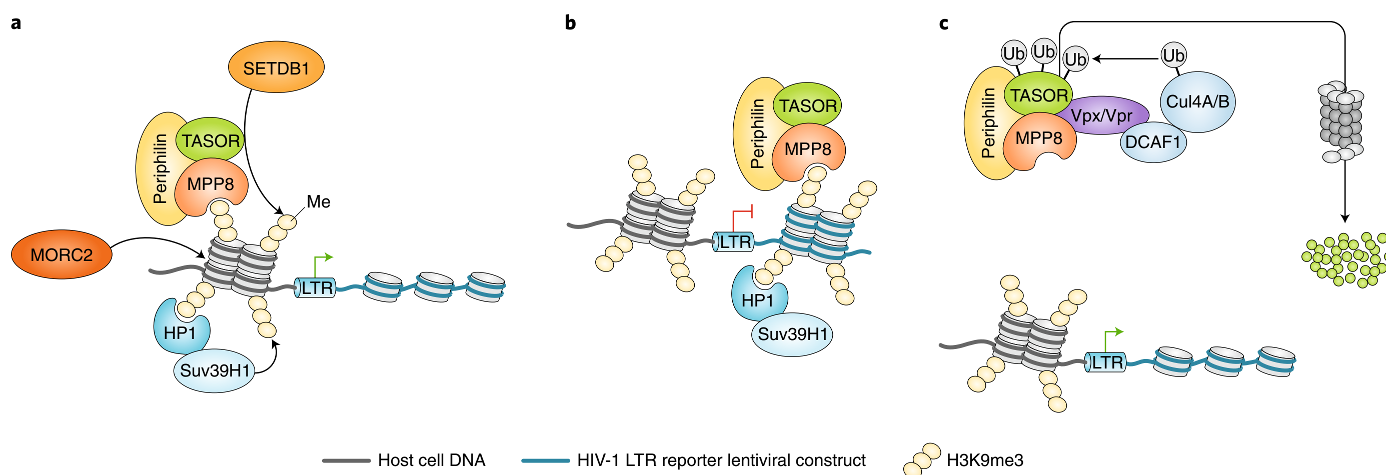

Fig. 1 | Model for HUSH-mediated epigenetic silencing of HIV-1 transcription during latency and Vpx/Vpr-mediated reactivation through HUSH complex degradation. a,**b**, It has been previously reported that HP1 and the histone methyltransferase Suv39H1 respectively binds and deposits the repressive H3K9me3 on the HIV-1 LTR promoter[14,15], thereby contributing to viral transcriptional silencing. Chougui et al.[4] and Yurkovetskiy et al.[5] suggest that the HUSH complex (composed of TASOR, MPP8 and periphilin) could also be involved in H3K9me3-mediated transcriptional repression of the HIV-1 provirus. The chromodomain of MPP8 binds the H3K9me3 histone mark and is thus required for the initial targeting of the HUSH complex to genomic loci rich in H3K9me3 (**a**), where subsequent recruitment of the histone methyltransferase SETDB1 is required for further H3K9me3 deposition to spread epigenetic transcriptional silencing (**b**). **c**, The viral protein Vpx/Vpr associates with TASOR and induces its ubiquitination through the recruitment of the DCAF1 adaptor for the Cul4A/B ubiquitin E3 ligase complex and its subsequent proteasomal degradation. As consequence, Vpx/Vpr expression phenocopies TASOR depletion and causes a decrease in H3K9me3 levels and derepression of silent proviruses in HIV-1 latency cellular models.

SETDB1, HUSH also recruits the chromatin-remodelling factor MORC2, and MORC2 ATPase activity is required to mediate epigenetic silencing[8]. Earlier studies have shown that HUSH depletion activates transcription from newly integrated retroviral or HIV-1 vectors, retrotransposons and transposable elements from the long interspersed element-1 class[6–8].

To find new targets of SIV Vpx-mediated degradation, Chougui et al.[4] used a large-scale SILAC (stable-isotope labelling by amino acids in cell culture)-based quantitative proteomic screen and identified, among more than 1,500 quantified nuclear proteins, only 30 proteins as preferential cellular targets of Vpx, including TASOR and MPP8 (in addition to SAMHD1). In this issue of *Nature Microbiology*, Yurkovetskiy et al.[5] first observed that Vpx and Vpr encoded by diverse primate immunodeficiency viruses activated transcription from repressed proviruses. Then, to gain insight into the mechanisms by which Vpx overcomes transcriptional repression of lentiviral transgenes, Yurkovetskiy et al.[5] next performed a loss-of-function screen, focusing on genes previously reported to contribute to silencing of retroviruses and other transcriptional targets. Similar to the proteomic screen reported by Chougui et al.[4], this short-hairpin-RNA-based screen allowed Yurkovetskiy et al.[5] to identify the three HUSH complex components (TASOR, MPP8 and periphilin), as well as the associated MORC2, as transcriptional silencing factors of proviral reporter gene expression. In a series of elegant and well-controlled experiments, Chougui et al.[4] confirmed TASOR downregulation by Vpx in primary CD4$^+$ T cells and macrophages and in HIV-2-infected cells, and showed that this required proteasome activity. Silencing DCAF1 or blocking Vpx binding to DCAF1 or to TASOR impaired TASOR but not SAMDH1 degradation, showing that TASOR and SAMDH1 degradations are two genetically separable events. Yurkovetskiy et al.[5] also demonstrated a reduction in the steady-state level of all three core HUSH complex components in response to Vpx, but in contrast a concomitant increase in MORC2 protein expression level was shown[5].

Both studies showed that, although antagonism of human HUSH was not conserved among all human and simian *vpx* and *vpr* genes tested (notably HIV-1)[4,5], Vpr proteins from African green monkey SIV and from the phylogenetic divergent l'Hoest's monkey SIV degraded TASOR, arguing in favour of an ancient species-specific common function of Vpx/Vpr in primate lentiviruses. However, the two groups observed differences among the Vpx/Vpr proteins from human and simian lineages in terms of TASOR degradation and/or transactivation activities of HUSH-repressed promoters. These discrepancies are likely due to viral isolate-specific differences. Indeed, Vpx and Vpr sequence variability is among the highest observed in lentiviral coding sequences[9]. Technical variations in lentiviral Vpx and Vpr protein expression (by in *trans* delivery through virus-like particles or by transduction with a lentiviral vector) might account for the differences between the two studies. Further work will be therefore needed to obtain a functional and phylogenetic overview of the divergent lentiviral Vpx/Vpr proteins and their ability to counteract HUSH-mediated silencing. Nevertheless, both studies highlight a pivotal role of these accessory proteins in epigenetic control of all HUSH-regulated genes and thus might be relevant beyond the field of HIV and SIV.

The previous report by Tchasovnikarova et al.[6] had demonstrated that HUSH derepresses HIV-1 transcription in the J-LatA1 cell clone, a Jurkat T-cell-derived HIV-1 latency model bearing a unique copy of a reporter HIV-1 long terminal repeat-green fluorescent protein (LTR-GFP) mini-genome that has been stably integrated and epigenetically silenced[10]. Both *Nature Microbiology* studies[4,5] investigated the effects of Vpx/Vpr-mediated HUSH degradation on repressed integrated HIV-1 LTR-GFP reporter constructs and observed increased GFP expression[4,5] and loss of proviral H3K9me3 marks[4] in response either to Vpx/Vpr delivery[4] or to TASOR silencing[5], indicative of transcriptional reactivation of the repressed HIV mini-genome and in agreement with previous work[6]. Similar results were obtained using the Jurkat T-cell line clone J-Lat A7, but not the J-Lat A2 clone[4], consistent with the observation that HUSH mediates position-effect variegation[6]. The Vpx Q76R DCAF1-binding-deficient mutant and Vpx Q47A/V48A TASOR-binding-deficient mutant failed to reactivate proviral transcription[4], suggesting that the ability of Vpx to transactivate latent HIV-1 LTRs correlates with its capacity to destabilize HUSH. Importantly, Yurkovetskiy et al.[5] also evaluated the effect of Vpx/Vpr on a polyclonal population of cells carrying the reporter HIV-1 mini-genome integrated at many positions and confirmed that diverse Vpx/Vpr proteins as well as knockdown of TASOR or of SETDB1 counteracted the HUSH-mediated transcriptional repression of the HIV miniproviruses[5]. The same authors next showed that, in the context of a spreading infection, replication kinetics of HIV-1 and rhesus macaque SIV were accelerated to a similar extent by *vpx* or TASOR knockdown[5].

Taken together, these two studies confirm the previous work by Tchasovnikarova et al.[6] suggesting that the HUSH complex could constitute a new epigenetic silencing mechanism of HIV-1 latency and therefore a new potential molecular target for anti-latency strategies. Further research is needed to ascertain the role of Vpx/Vpr-mediated HUSH downregulation in reactivation of latent HIV, notably by using full-length HIV proviruses, primary cell models of HIV latency and cells isolated from HIV$^+$ individuals. The persistence of latent reservoirs harbouring transcriptionally silent HIV proviruses is a major barrier to curing HIV infection. Post-integration latency is a multifactorial phenomenon involving various transcriptional and post-transcriptional blocks[11]. Among these, epigenetic modifications, such as histone methylation, are critical in establishing and maintaining HIV proviruses in a latent state. Three histone methyltransferases (HMTs) have been previously reported to contribute to HIV-1 transcriptional silencing in HIV-1 latently infected cells[12–16]. EHMT1/GLP and EHMT2/G9a participate in HIV-1 latency by depositing dimethylation on lysine 9 of histone H3 (H3K9me2) on the HIV-1 promoter[12,13,16]. The chromodomain protein heterochromatin protein 1 (HP1) recognizes H3K9me3 and further recruits the HMT Suv39H1, which deposits repressive H3K9me3 histone marks on the HIV-1 promoter (Fig. 1), thereby contributing to viral transcriptional silencing[14,15]. The HP1/Suv39H1 system triggers a positive feedback loop responsible for spreading of heterochromatin along a given DNA region. Interestingly, the HUSH complex also binds to H3K9me3 and recruits a HMT (SETDB1) for further deposition of H3K9me3, and thus shows mechanistic similarities to the HP1/Suv39H1 system. This observation raises questions about the nature of the biological conditions determining whether HIV proviruses will be subject to HUSH/SETDB1- or HP1/Suv39H1-mediated silencing, and about the possible interplay between these two epigenetic mechanisms. In addition, with HUSH antagonism absent in HIV-1 Vpr, it remains to be determined how silencing by HUSH is counteracted in HIV-1. The answers to these questions will provide not only new insights into viral pathogenesis, but also new therapeutic opportunities to either lock out or reactivate

latent proviruses in HIV reservoirs of infected individuals.

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Competing interests

The author declares no competing interests.