Amino acid starvation induces reactivation of silenced transgenes and latent HIV-1 provirus via down-regulation of histone deacetylase 4 (HDAC4)

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AUTHOR SUMMARY

Mammalian cells protect themselves from invasion of their genomes by silencing integrated nonnative DNA sequences (or transgenes), such as retroviral genomes and other parasitic elements. However, this physiological response can exert an inhibitory influence on gene therapy by inhibiting the expression of therapeutic transgenes (1), or it can allow human pathogens, such as HIV-1, to escape immunological or pharmacological control (2). Here, we show that depriving cells of essential amino acids that are necessary for protein synthesis results in the transcriptional derepression of silenced transgenes by a mechanism involving the enzyme histone deacetylase 4 (HDAC4), thereby presenting researchers with a target relevant for enhancing gene replacement and antiretroviral therapies.

The present study originated from a serendipitous and apparently counterintuitive observation. Indeed, we found that exogenous proteins accumulated at remarkably high levels when human HeLa epithelial cells carrying an integrated transgene, such as a plasmid or a retroviral expression vector, were cultivated in the absence of essential amino acids (Fig. P1, Upper). This phenomenon was specific for the exogenous proteins regardless of whether they were expressed under the control of a viral or human promoter sequence. In contrast, the corresponding endogenous counterparts, physiologically encoded by the host cell genome, were either not affected or down-regulated. To dissect the mechanism involved, we first tested the mammalian target of rapamycin and stress-activated protein kinase signaling pathways, which are known to be sensitive to amino acid starvation. However, this effort yielded negative results. Next, we reasoned that amino acid deprivation might affect gene expression by epigenetic modifications of DNA or histones, which belong to the main family of proteins that associate with DNA. We found that although inhibitors of DNA methylation did not affect transgene expression, inhibition of histone deacetylation closely reproduced the effects of starvation.

Considering these results, we analyzed the full repertoire of genes expressed in the cells before and after amino acid deprivation, and, interestingly enough, we found that the second-most strongly down-regulated gene on starvation was HDAC4. Indeed, we demonstrated that HDAC4 expression was inversely correlated to the expression of the transgene; HDAC4 was clearly detectable under normal conditions in which the transgene expression was repressed, and its level was very low or undetectable under starvation when transgene expression was up-regulated. The role of HDAC4 was confirmed by suppressing its activity by using either specific pharmacological inhibitors or RNAi. The results showed that the specific loss of HDAC4 activity is sufficient to up-regulate transgene expression, although at lower levels than the


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levels under amino acid starvation, suggesting that HDAC4 is a crucial player in this process.

Among the possible applications of these findings, we tested whether this pathway could also affect the expression of the genome of HIV-1, the etiological agent of the AIDS syndrome, in latently infected cells. HIV-1 is a human lentivirus that infects T lymphocytes and monocytes, and integrates into the cell genome as part of its life cycle. In addition to acute virus replication, in a minority of infected cells, HIV-1 becomes transcriptionally “dormant,” making these cells a reservoir insensitive to the antiviral effects of combined antiretroviral therapy (cART). This pool of latently infected cells is believed to play a crucial role in virus reactivation on suspension of cART, and several strategies to remove these cells are under investigation. Because histone modifications have been implicated in maintaining HIV-1 latency, HDAC inhibitors represent potential tools in combination with cART for the objective of purging the viral reservoirs in infected individuals (3). Using two well-characterized cellular models of latent HIV-1 infection, we demonstrated that either amino acid starvation or pharmacological inhibition of HDAC4 induced the reactivation of HIV-1 transcription and virus production in the ACH-2 T-lymphocytic cell line (Fig. P1, Lower), which expresses HDAC4, but not in the U1 promonocytic cell line, which does not express the enzyme. In conclusion, these findings suggest that selective HDAC4 inhibitors, such as MC1568, might represent an attractive possibility, compared with the more toxic broad-spectrum HDAC inhibitors, in strategies aimed at either controlling the expression levels of therapeutic transgenes or eradicating dormant HIV-1 from latently infected cells without significant cytotoxicity.