UHRF1 regulates multiple layers of HIV-1 expression, from epigenetics to post-transcriptional events

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BACKGROUND

HIV-1 infects CD4$^+$ cells, primarily CD4$^+$ T cells but also monocyte-macrophages. Afte infection, its genome integrate into the host cell on and in some case can enter latency, a state of minimal transcription arising from the 5′ LTR promoter. HIV-1 latency and persistence arise from multiple layers of transcriptional and post-transcriptional repression that include proviral integration into transcriptionally silent chromatin, recruitment of histone deacetylases and methyltransferases to maintain a closed chromatin state, limited availability of Tat and P-TEFb to drive transcriptional elongation, and barriers to HIV RNA splicing, nuclear export, and translation. In addition, inducible transcription factors such as NFAT, AP-1, and NF-κB are often scarce or sequestered in resting CD4$^+$ T cells, further restricting viral transcription initiation. Non-T cell reservoirs and patient-specific factors, including integration site, cell subset, and immune microenvironment, compound these challenges. The “shock and kill” strategy leverages latency-reversing agents (LRAs) to overcome these molecular blocks and expose infected cells to immune-mediated clearance. To date, preclinical and clinical studies with LRAs have failed to meaningfully reduce reservoir size, reflecting their limited ability to overcome both transcriptional elongation and post-transcriptional blocks. **A deeper understanding of the full molecular mechanisms governing HIV-1 latency is therefore critical to identify novel targets and develop more potent latency reversal approaches.**

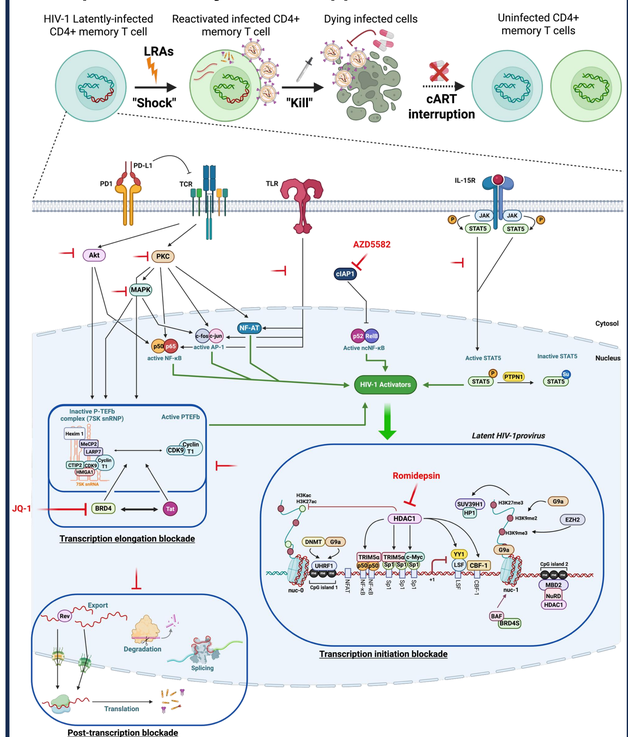


Figure 1 – mechanisms of HIV-1 persistence and cure strategies.

RESULTS

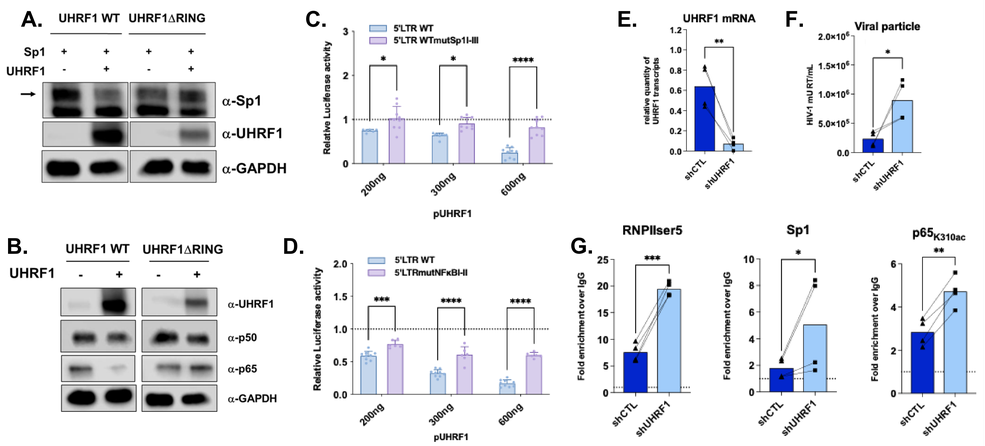


Figure 2 – UHRF1 represses HIV-1 transcriptional initiation by mediating proteasomal degradation of Sp1 and NF-kB

(A)-(B) HEK-293T cells were transiently co-transfected or not with a dose of an expression vector for UHRF1WT or lacking its RING domain and an expression vector for either Sp1 or either p50 and p65. Protein levels of UHRF1, Sp1, p50, p65, or GAPDH were assessed by immunoblot in nuclear extracts. **(C)-(D)** HEK-293T cells were transiently co-transfected with either luciferase reporter construct containing either a WT 5’LTR promoter or mutated for either the three Sp1 binding sites (5’LTRmutSp1(I)-(III)) or either the two NF-kB binding sites (5’LTRmutNF-kBI-II) of the core promoter sequence and increasing doses of a pUHRF1 expression vector. After 48H, cells were harvested, lysed, the firefly luciferase activity was quantified and first normalized to protein concentration and then normalized to the mock luciferase value. **(E)** Jurkat HIVGKO cells were shCT or shUHRF1 transduced and, after puromycin selection, UHRF1 downregulation was validated by RT-qPCR and **(F)** HIV-1 particle production was assessed by SG-PERT. **(G)** Chromatin samples were prepared and immunoprecipitated using either a non-specific IgG (serving as negative control) or antibodies recognizing either RNPIIser5, either Sp1 or P65K310ac. Precipitated DNA was purified and used for quantification of HIV DNA by qPCR using primers targeting the 5’ LTR. For all panels, one representative experiment out of 3 is presented. The significance of the results was assessed by a paired Student test: * p-value ≤ 0.05, ** p-value ≤ 0.01, **** p-value ≤ 0.0001 and ns = not significant.

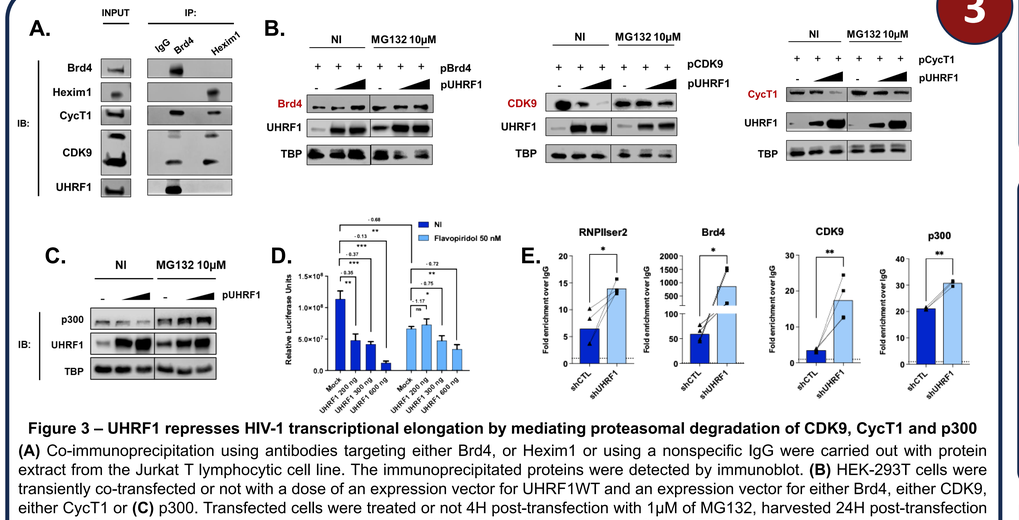


Figure 3 – UHRF1 represses HIV-1 transcriptional elongation by mediating proteasomal degradation of CDK9, CycT1 and p300

(A) Co-immunoprecipitation using antibodies targeting either Brd4, or Hexim1 or using a nonspecific IgG were carried out with protein extract from the Jurkat T lymphocytic cell line. The immunoprecipitated proteins were detected by immunoblot. **(B)** HEK-293T cells were transiently co-transfected or not with a dose of an expression vector for UHRF1WT and an expression vector for either Brd4, either CDK9, either CycT1 or **(C)** p300. Transfected cells were treated or not 4H post-transfection with 1μM of MG132, harvested 24H post-transfection and used for total protein extraction. Protein levels of UHRF1, Brd4, CDK9, CycT1, p300 or TBP (serving as a loading control) were assessed by immunoblot in nuclear extracts. **(D)** HEK293T were treated or not with 50 nM of flavopiridol for 24H before transfection with an increasing dose of UHRF1 overexpression vector along with a reporter luciferase construct for the 5’LTR promoter activity. Luciferase activity was quantified as described in figure 2. **(E)** Chromatin samples from the experiment of Fig2E-G were prepared and immunoprecipitated using either a non-specific IgG (serving as negative control) or antibodies recognizing either RNPIIser2, either Brd4, either CDK9 or p300. Precipitated DNA was purified and used for quantification of HIV DNA by qPCR using primers targeting the 5’ LTR. For all panels, one representative experiment out of 3 is presented. The significance of the results was assessed by a paired Student test: * p-value ≤ 0.05, ** p-value ≤ 0.01, **** p-value ≤ 0.0001 and ns = not significant

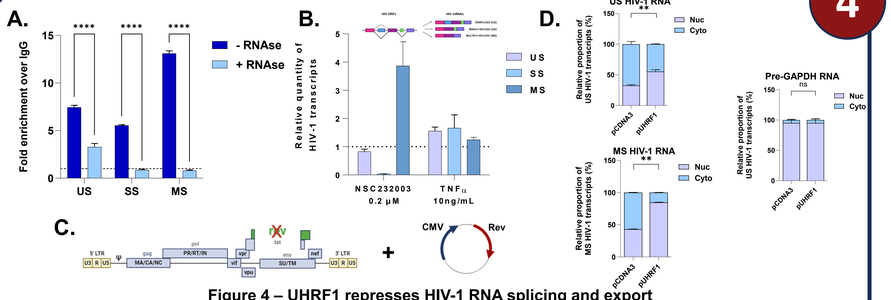


Figure 4 – UHRF1 represses HIV-1 RNA splicing and export

(A) HEK293T cells were co-transfected with the HIV pNL4.3 strain and 48H post-transfection cells were harvested, fixed with formaldehyde and sonicated before using the resulting extracts in immunoprecipitation experiments using a non-specific IgG or an antibody targeting UHRF1 in presence or not of RNAse I. The immunoprecipitated RNA was purified and assayed for the different types of HIV RNA by RTqPCR using primers targeting US, SS, or MS RNA. **(B)** Primary CD4+T cells were infected with HIV and 72H post-infection, cells were treated or not with 200μM of NSC232003. After 72h cells were harvested and the RNA was extracted and assayed for the different HIV RNA as explained in (A). **(C)** Schematic representation of the experimental plan of the experiment in (D). **(D)** HEK293T cells were co-transfected with the HIV pNL4.3 strain and an overexpression vector for UHRF1WT or not. 48H post-transfection cells were harvested and fractionated into nuclear and cytoplasmic fractions using a hypotonic buffer. RNA from each fraction was extracted and and assayed for the different HIV RNA as explained in (A). For all panels, one representative experiment out of three is presented. The significance of the results was assessed by a paired Student test: * p-value ≤ 0.05, ** p-value ≤ 0.01, **** p-value ≤ 0.0001 and ns = not significant.

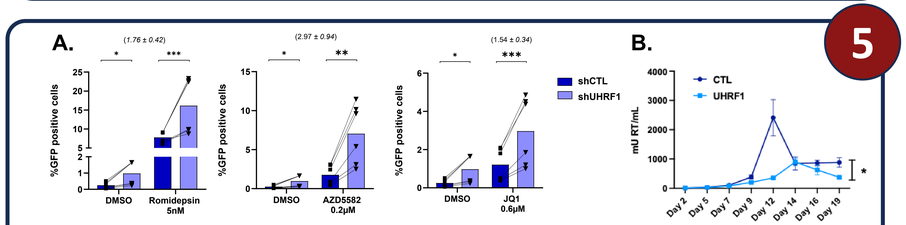
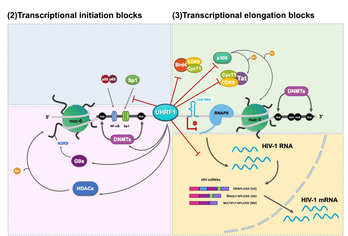


Figure 5 – UHRF1 overexpression represses HIV-1 replication, and its downregulation potentiate HIV latency reversal.

(A) Jurkat HIVGKO were either shCT- or shUHRF1 transduced and, after puromycin selection cells were mock treated or treated with either Romidepsin , either AZD5582 or JQ1. 24hours after treatment percentages of GFP+ cells were measured by flow cytometry. **(B)** Jurkat T cells constitutively overexpressing UHRF1 or not (CTL) were generated by lentiviral transduction. Following selection, transduced cells were infected with a fully replicative strain of HIV-1, cultured for 20 days. Culture supernatents were harvested every two days used for viral particle quantification by SG-PERT at the end of the experiment. For all panels, one representative experiment out of three is presented. The significance of the results was assessed by a paired Student test: * p-value ≤ 0.05, ** p-value ≤ 0.01, **** p-value ≤ 0.0001 and ns = not significant.

TAKE HOME MESSAGE



Our data reveal an important role for the cellular protein UHRF1 in HIV-1 silencing, acting at multiple regulatory checkpoints by maintaining histone hypermethylation in the 5’LTR (epigenetic), promoting the proteasomal degradation of Sp1 and NF-kB (transcription-initiation) as well as of the active P-TEFb complex (transcription-elongation) and inhibiting HIV-1 RNA splicing and export (post-transcription). Moreover, our findings demonstrate that molecular downregulation of UHRF1 expression potentiates the activity of several LRAs in latently-infected T cells. Our findings highlight the multiple repressive mechanisms used by UHRF1 and reinforce its relevance as an attractive therapeutic target for anti-HIV cure strategies.

PLAIN LANGUAGE SUMMARY

We showed that the cellular protein UHRF1 maintains HIV-1 silent at several stages, and reducing UHRF1 makes it easier to reactivate the virus and improve the effectiveness of drugs aimed at clearing latent infection.

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