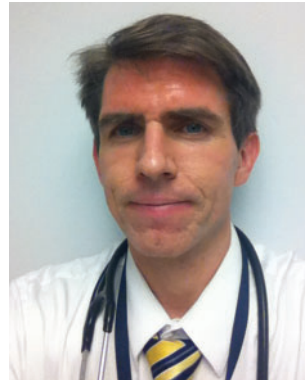


Exploring HIV latency using transcription profiling



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The major barrier to a cure for HIV is the existence of reservoirs consisting predominantly of latently infected CD4⁺ T cells, which do not produce virus constitutively but can be induced to produce infectious virus on activation. HIV latency research has largely focused on peripheral blood, yet most HIV-infected cells reside in tissues, especially the gut, where differences in drug penetration, cell types, and immune responses may impact mechanisms of persistence. Exploring the differences between the gut and the blood in transcriptional blocks may reveal fundamental insights into mechanisms that contribute to HIV latency. Our novel transcriptional profiling assays enable us to determine where blocks to HIV transcription occur in various tissues and the magnitude of their contribution. These assays could also be adapted to investigate latency established by other retroviridae or even DNA viruses such as herpesviridae with a view to pinpointing mechanisms underlying latency *in vivo* and ultimately contribute to designing a cure.

Probing HIV in the gut

HIV remains a major pandemic, with more than 36 million people affected worldwide. Over 1.1 million people in the US are currently living with HIV. In Australia, increased awareness and high profile health promotion campaigns have been unsuccessful in reducing the number of new infections, which have remained steady over the last few years¹. Despite the success of combination antiretroviral therapy (ART) in suppressing HIV-1 replication, ART is not curative and residual virus continues to cause immune activation, organ damage, and reduction in life expectancy^{2,3}. HIV-1 evades ART and immune responses through latent infection of CD4⁺ T cells⁴⁻⁷. Since these latently infected cells do not produce HIV proteins, they

escape viral cytopathic effects and evade detection by the immune system. Latent HIV has been primarily found in long-lived memory CD4⁺ T cells, which can survive for decades and expand the viral reservoir by cell proliferation⁸⁻¹¹. These latently infected cells are considered to be the main barrier to HIV eradication⁴ and their reactivation *in vivo* likely contributes to sustained immune activation observed during suppressive ART¹².

Although much HIV latency research utilises *in vitro* models or cells from peripheral blood, prior work has highlighted differences between the gut and blood in the phenotype of infected T and non-T cells^{12,13}. Furthermore, gut and blood compartments differ in levels of T cell activation and its relationship with HIV transcription¹². Considering that the gut harbors up to 85% of all lymphoid tissue and over 90% of all lymphocytes^{14,15}, it is imperative to investigate how mechanisms of HIV persistence and latency differ between gut and blood *in vivo*. To this end, we are employing a cutting-edge 'transcription profiling' approach, which features a novel panel of highly conserved, sensitive, quantitative reverse transcription droplet digital PCR (RT-ddPCR) assays. This approach quantifies the levels of HIV transcripts that suggest different mechanisms of transcriptional blockade and/or progression through various stages of HIV transcription. The levels and ratios of different HIV transcripts can be used to determine the degree to which different mechanisms contribute to reversible inhibition of HIV gene expression, and hence latency, in cells from HIV-infected individuals.

Exploiting transcriptional features of HIV

The compact genome of HIV features major coding regions, including: 1) *gag*, *pol* and *env*, common to all retroviruses, which

encode essential structural proteins (such as envelope, matrix and capsid) and critical enzymes, including protease (catalyses cleavage), reverse transcriptase (reverse transcribes RNA genome into double-stranded DNA) and integrase (mediates integration into host genome); 2) regulatory genes (*tat* and *rev*); and 3) accessory genes (*vif*, *vpr*, *vpu* and *nef*) (Figure 1). A major putative mechanism driving HIV latency is transcriptional interference (TI), caused by ongoing transcription of host genes *in cis* that inhibit the assembly of the RNA polymerase complex on the HIV promoter region, the 5'-long terminal repeat (LTR)^{16–19}. 'Read-through' transcripts (Figure 1) are suggestive of TI since they include the U3-U5 region that distinguishes them from canonical HIV transcripts. Other mechanisms that can lead to a block to HIV transcription initiation include epigenetic modification, a lack of host initiation factors^{18,20}, suboptimal activity of the viral transcription factor Tat²¹ and integration into transcriptionally silent regions of the genome^{18,22,23}. The degree of transcriptional initiation can be assessed by detection of transcripts containing the 'transactivation response' (TAR) element, which is the RNA target of Tat protein and is present in all HIV transcripts (Figure 1). Our 'TAR' assay has been specifically designed to maximise the detection of short, prematurely terminated transcripts with an efficiency equal to longer transcripts^{24,25} by incorporating an additional polyadenylation step that generates an accessible priming site for reverse transcription. This strategy offers a considerable advantage over other assays, which can detect only 4% of true short transcripts and thus significantly underestimate the abundance of these transcripts²⁴.

Other proposed mechanisms of HIV latency include downstream blocks to elongation due to the lack of host elongation factors, the presence of inhibitory factors, nucleosome conformation and

insufficient Tat activity^{21,26–28}. Such mechanisms can be evaluated by targets for downstream sequences (such as 'Long LTR') that indicate elongation past the TAR loop. To assess how efficiently transcription proceeds through *pol* to the 3' end, transcripts containing *pol* and *nef* target sequence are also detected by our panel of assays. Levels of polyadenylated HIV RNA ('PolyA'), indicative of transcription completion²⁹, are detected using primers that span the LTR (U3) and polyA tail. Polyadenylated transcripts can act as surrogate markers for HIV protein. Similarly, multiply spliced HIV RNA ('Tat-Rev'), heralding the completion of splicing, can serve as a surrogate for productive infection³⁰. The levels of each distinct transcript and the ratios between them can be used to quantify the degree to which HIV transcription is inhibited *in vivo* by TI or blocks to transcriptional initiation, elongation, completion and splicing.

The novelty of this approach lies in the ability to simultaneously investigate multiple mechanisms of transcriptional blocks *in vivo*. Combined with RT-ddPCR, which enables absolute cDNA quantification²⁴, this approach provides a considerable advantage over previous work that mostly focuses on one mechanism of latency at a time and has typically utilised *in vitro* models of latency, which may not recapitulate what happens *in vivo*.^{18–20,22,23,26–28} Unlike previously employed strategies, specific blocks to transcription and the magnitude of their impact on the prevailing levels of HIV RNA can be simultaneously assessed by determining the expression of these processive transcripts. These data can then inform strategies to target latency reversal. Using matched tissues from ART-suppressed HIV-infected individuals, this transcription profiling approach is beginning to reveal differences between blood and gut in the blocks to HIV transcription, which is of particular interest due to the difficulty in accessing tissue samples and the subsequent

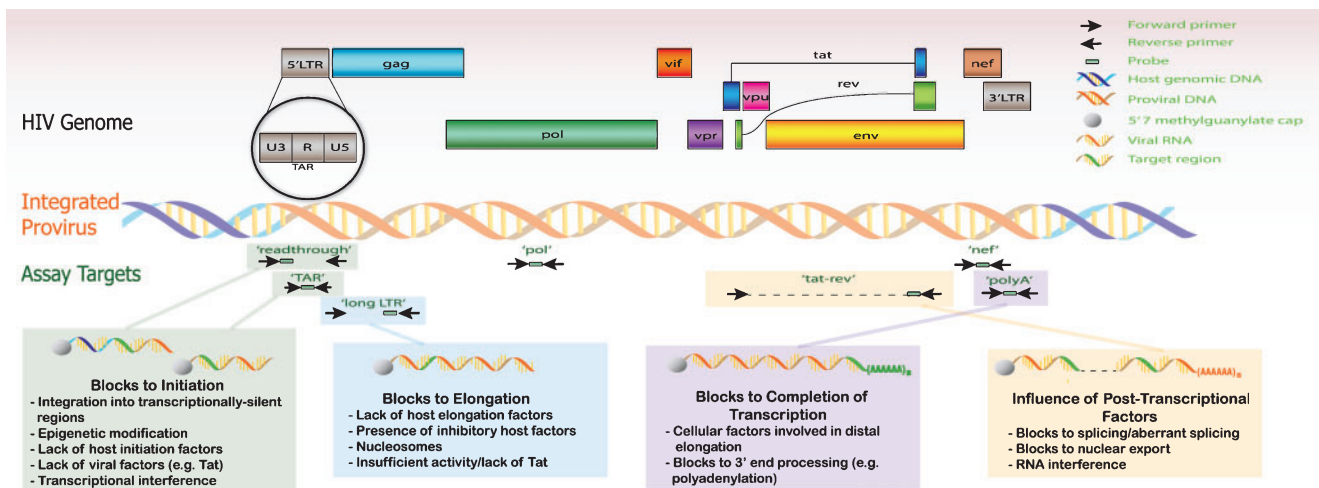


Figure 1. The HIV genome and the targets for transcription profiling assays. This schematic represents the genetic organisation of proviral HIV DNA and the HIV RNA assays that target specific sequence regions, which reveal insight into blocks to transcription. Some proposed mechanisms that underlie the blocks to transcription initiation and elongation are listed.

paucity of data examining HIV latency in the gut. This work, which contributes to elucidating the molecular mechanisms that govern HIV latency, may lead to new therapies aimed at curing HIV.

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Biographies

Sushama Telwatte is a Postdoctoral Scholar undertaking postdoctoral studies in the laboratory of Associate Professor Steven A Yukl at the Veteran Affairs Medical Center in San Francisco. Her work focuses on uncovering the mechanisms underlying HIV persistence and latency in the gut and blood *in vivo*. Sushama completed her PhD under the supervision of Associate Professor Gilda Tachedjian at the Burnet Institute/Monash University, investigating the role of synonymous mutations in HIV-1 selected during drug therapy. Sushama was awarded the ASM BD Award (Victorian Branch) and presented her PhD work at the ASM 2015 meeting.

Dr Steven Yukl is an Associate Professor of Medicine at the University of California, San Francisco (UCSF) and a staff physician at the San Francisco Veterans Affairs Medical Center. His research focuses on the mechanisms that allow HIV to persist despite immune defenses and antiretrovirals, thereby preventing HIV cure. Dr Yukl has been involved in basic, translational, and clinical research on HIV persistence since 2005, with specialisation in HIV latency, gut tissue reservoirs, cellular reservoirs, and development of ultrasensitive assays to measure HIV persistence.