Transient-mediated fate determination in a transcriptional circuit of HIV

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4 Figures

Steady-state behavior and bistability have been proposed as mechanisms for decision-making in gene circuits[1-3]. However, transient gene expression has also been proposed to control cell fate[4, 5] with the decision arbitrated by the lifetime of the expression transient. Here, we report that transcriptional positive-feedback plays a critical role in determining HIV infected cell-fate by extending the duration of Tat expression transients[6, 7] far beyond what protein half-life modulation can achieve. To directly quantify feedback strength and its effects on the duration of Tat transcriptional pulses, we exploit the noise inherent to gene-expression and measure shifts in the autocorrelation of expression noise. The results indicate that transcriptional positive-feedback extends the single-cell Tat expression lifetime by ~6-fold for both minimal Tat circuits and full-length, actively-replicating HIV-1. Importantly, artificial weakening of Tat positive-feedback shortened the duration of Tat expression transients and biased the probability in favor of latency. Thus, transcriptional positive-feedback appears to modulate transient expression lifetime and thereby control cell-fate in HIV.

Upon infecting a $CD4^+$ T lymphocyte, the Human Immunodeficiency Virus type 1 (HIV-1) can enter one of two developmental fates: active replication (lysis) or proviral latency (an analog of phage lysogeny). The vast majority of infections lead to active replication, destroying the T-cell in ~40hrs and producing many hundreds of infectious viral progeny[8, 9]. A small minority of infections enter proviral latency, a long-lived quiescent state where viral gene expression is turned off[10, 11]. Both developmental fates

are clinically relevant: active HIV replication destroys the immune system and eventually causes AIDS, while latently-infected $CD4^+$ T-lymphocytes are the major reservoir thwarting HIV-1 eradication from the patient[12]. While many host factors have been implicated in controlling HIV-1 replication and latency[13-15], the HIV-1 Tat protein (Trans-Activator of Transcription) is absolutely essential for active replication and latent reactivation[13, 16-18]. Tat transactivation drives active replication by mediating hyper-phosphorylation of RNA polymerase II to enhance transcriptional elongation from HIV's Long-Terminal Repeat (LTR) promoter[6, 13, 19, 20]. Tat transactivation thus comprises an essential positive-feedback loop that drives HIV lytic replication by auto-stimulating its own gene expression 50-100 fold above basal levels and simultaneously up-regulating the expression of HIV Rev (the essential viral mRNA export factor) and Nef [21]. We recently reported the existence of a Tat *feedback-resistor* that drives Tat expression pulses which decay to a monostable off state and stabilizes latency[6]. However, it was not clear how a circuit that is monostable for one fate (latency) could act as a switch between two cell fates (proviral latency vs. active replication). Here we test if positive-feedback can modulate the duration of expression transients and thereby mediate a decision between active replication and latency (Fig 1). Specifically, we hypothesized that relatively strong positive-feedback generates long duration Tat transcriptional pulses, which should drive lytic replication and destroy the infected T-lymphocyte before the Tat transient decays back to the off state. Conversely, weaker positive feedback would generate shorter transcriptional pulses, which may bias the probability in favor of latency.

To determine if positive-feedback modulated the Tat expression transient, we *directly* measured positive-feedback strength via fluctuation autocorrelation analysis and

calculated the degree of Tat expression pulse extension as described below. We utilized a recently developed gene expression fluctuation autocorrelation theory[22, 23] which allows convenient analysis of feedback strength via noise autocorrelation functions (ACF). While the noise structure of transcriptional positive-feedback has not been previously measured, positive-feedback is predicted to shift the noise ACF to longer times (i.e. increase the duration of stochastic fluctuations) with a magnitude related directly to the feedback strength. Heuristically, this prediction can be understood by comparing time-series data for minimal HIV circuits with LTR driving GFP (LTR-GFP; no feedback) or GFP and Tat (LTR-GFP-Tat; positive feedback) expression (Fig. 1D).

Starting with observations of GFP fluorescence from individually tracked single cells (indexed by i, where i = 1,2...total number of cells tracked) we define noise functions, $N_i(t)$, where the deterministic components (basal and transient) of expression are removed, noise magnitudes are scaled by the total magnitude of expression, and the baselines are suppressed (i.e. the $N_i(t)$ functions are zero mean) for the duration of the observation (Supplementary Information). As the duration of observation are by necessity time limited, the $N_i(t)$ functions are missing low-frequency components of the noise. However, we derived normalized high-frequency ACFs (referred to as ACFs in the remainder of the text, see Supplementary Information) as $\Phi_i(t) = \langle N_i(t) \bullet N_i(t+\tau) \rangle / \langle N_i(t)^2 \rangle$ where $\langle \cdot \rangle$ represents the average, τ varies between 0 and ∞ (i.e. $\Phi_i(0)=1$ and $\Phi_i(\infty)=0$; perfect correlation at $\tau=0$ and completely uncorrelated at $\tau=\infty$). Composite ACFs were found by averaging individual cell ACFs over the entire population of tracked cells, and shifts in the feedback strength, T, were found from a comparison between $\tau_{1/2}$ values ($\Phi_i(\tau_{1/2}) = 0.5$) for feedback (FB) and non-feedback

cases (nonFB). $T \to 1-\left(\tau_{1/2\_nonFB}/\tau_{1/2\_FB}\right)$, where $\to$ represents an equality for true ACFs[23] and represents a mapping operator for high frequency ACFs (Supplementary Information). Negative values of T indicate negative feedback and positive values indicate positive feedback which will increase the ACF $\tau_{1/2}$ ($\tau_{1/2\_FB} > \tau_{1/2\_nonFB}$). Similarly, positive feedback also extends the duration of transient excursions by $1/(1-T)$[22, 23].

Feedback strength was measured in a minimal HIV LTR-GFP-Tat circuit[7] from single-cell gene-expression (i.e. GFP intensity) fluctuations (Fig. 2A). Noise ACFs for the LTR-GFP-Tat circuit and a non-feedback LTR-GFP control circuit were compared to minimize the effect of non-biological (i.e. instrumental) noise in both the absence or presence of exogenous Tat protein stimulation (Fig. 2B-C) and tumor necrosis factor α stimulation (TNFα, Supplementary Information) [24-26]. In all cases the measured shift in ACF shows that Tat positive-feedback increases the duration of transient Tat expression pulses by at least 60% and possibly as much 10-fold (Supplementary Information). Furthermore, down-modulation of Tat positive-feedback by SirT1 over-expression, or using a previously characterized Tat mutant[6, 7] (K→A substitution at amino acid 50), led to significantly reduced feedback strength (Fig. 2D). Importantly, weaker positive-feedback correlated with a significantly quicker decay of the Tat expression transient (Fig. 2E). Cumulatively, these data experimentally validate our previous theoretical prediction[22, 23, 27] that positive-feedback increases the duration of gene-expression fluctuations, and demonstrate how positive-feedback extends the lifetime of transient pulses of gene expression.

Next, we measured how feedback strength correlated with Tat expression duration in both the minimal LTR-GFP-Tat circuit and a previously characterized full-length HIV-1 provirus[13, 19] containing GFP cloned in place of Nef (Fig. 3A-B). Since Tat, Rev, and Nef (now GFP) are alternatively spliced from one mRNA[28], GFP is a reporter for Tat in this system. Importantly, full-length HIV-1 exhibited positive-feedback strength similar to that found in the minimal LTR-GFP-Tat circuits (Fig. 3B). Time-lapse microscopy and flow cytometry then showed that the expression transient in the minimal LTR-GFP-Tat circuit continued to increase for ~30hrs while in full-length HIV-1 continued to increase for >40hrs (Fig. 3C). The half-life of these cells undergoing full-length lytic HIV-1 replication was determined to be $t_{1/2}$=39.5hrs (Fig. 3E), which is shorter than the duration of the Tat expression pulse and implies that Tat positive-feedback strongly biases infected cell fate in favor of lysis.

Next, to test if the Tat positive-feedback circuit acts as a probabilistic switch with stronger positive-feedback increasing the probability of lysis and weaker positive-feedback strength increasing probability of latency, we artificially weakened Tat positive-feedback strength by over-expressing SirT1 in the full-length HIV-1 system. Weakened positive-feedback strength in SirT1 over-expressing cells was confirmed by noise ACF analysis (Fig. 4A), and by serial increases in SirT1 over-expression which generated successive reductions in activated proviral gene expression (Supplementary information). SirT1 over-expressing cells with weakened Tat positive-feedback, exhibited significantly increased probability toward latency (Fig. 4B). These data support a model where Tat positive-feedback strength and the resulting transcriptional pulse mediate a probabilistic

switch whose outcome may be tuned by cellular modulation of feedback strength (e.g. SirT1 activity).

At its core, the architecture of this HIV Tat circuit is a transient pulse generator whose duration can be controlled by variable strength, non-latching, positive feedback over periods that greatly exceed cell division times. Importantly, expression transients mediated by long protein half-lives cannot achieve a similar type of modulation as the dilution effects of cell growth and division ultimately limit transient duration. Where dilution effects are especially significant, e.g. bacterial systems, similar positive-feedback pulse duration mechanisms may be used to tune cell fate determination, such as the recently reported *Bacillus subtilis* competence decision circuit[4, 5]. Circuit architecture can also impacts decision timing. In bistable circuits, such bacteriaphage λ lysis-lysogeny, the fate decision is made early while the execution occurs much later[29]. Conversely, circuits employing positive-feedback driven transients allow the fate decision to be distributed (i.e. integrated) over a much longer period of time, with decision and execution essentially happening simultaneously. Understanding the mechanisms underlying gene circuit and cell fate decisions may ultimately inform upon therapy strategies[30] and modulating Tat positive-feedback strength to bias the lysis-latency decision for therapeutic benefit may represent one such strategy.

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Figure Legends

Figure 1: Positive-feedback extends the lifetime of gene expression transients. **a**, The HIV-1 genome encodes the Tat positive-feedback circuit. This circuit is comprised of HIV-1 Tat which in its short-lived acetylated form (Tat_A) transactivates the viral promoter within the LTR but is also rapidly deacetylated by SirT1[6, 31]. HIV-infected T-cells undergoing active viral replication (i.e. with active Tat positive-feedback) have a average lifetime of ~40hrs[9]. **b**, Expression transients without positive-feedback are short-lived and die out quickly leading to latency (red). But, positive-feedback (in direct proportion to its strength or loop transmission) can extend the duration of gene expression[22] transients thereby favoring lytic replication (blue). **c.** Positive-feedback strength can be directly measured in single-cells by examining fluctuations in gene expression (left and middle) and calculating a fluctuation auto-correlation function (ACF, right). Positive-feedback shifts the ACF decay by a magnitude that correlates directly to the strength of positive-feedback[23].

Figure 2: Measuring positive-feedback strength by exploiting inherent gene expression noise. **a**, Single-cell time-lapse microscopy images of LTR-GFP-Tat Jurkat T-cells over 12hrs (left, images captured every 10mins), single-cell intensity (middle) and processed noise trajectories (right) for determining high frequency noise autocorrelation functions (ACFs). **b**, Measured ACFs for LTR-GFP-Tat (ACF $\tau_{1/2}$ = 1.59±0.08 hrs) and LTR-GFP control (ACF $\tau_{1/2}$ = 1.2±0.12 hrs); positive-feedback shifts HF-ACFs to longer

times. **c**, Measured ACFs after stimulation with exogenous Tat protein for LTR-GFP-Tat (ACF $\tau_{1/2}$ = 1.77±0.08 hrs) and LTR-GFP control (ACF $\tau_{1/2}$ = 1.37±0.10 hrs). **d**, Reducing feedback strength in LTR-GFP-Tat by over-expression of SirT1 (red diamond) decreases ACF shift (ACF $\tau_{1/2}$ = 1.54±0.07 hrs) compared to wild-type LTR-GFP-Tat circuit (blue diamond; ACF $\tau_{1/2}$ = 1.76±0.09 hrs). Measurements performed after stimulation of positive-feedback with TNFα. **e**, Flow cytometry measurement of decay from the Tat transactivated state in the LTR-GFP-Tat-K50A mutant circuit (red) vs. the wild-type LTR-GFP-Tat circuit (blue). 10^5 cells were sorted from the Tat transactivated (GFP+) state at time=0; SirT1 over-expression induces 6-fold quicker decay of the Tat expression pulse.

Figure 3: Positive-feedback strength drives an extended Tat expression transient in both minimal Tat circuits and full-length HIV-1. a-b. Noise ACF shift for LTR-GFP-Tat cells and full-length HIV-1 infected cells after TNFα induced reactivation. **c**, Time-lapse microscopy and flow cytometry (insets) for LTR-GFP-Tat (top) and full-length HIV-1 (bottom) after TNF activation show that expression continues to increase past 40hrs. **d.** Flow-cytometry live/dead analysis of full-length HIV-1 infected cells after activation by TNFα: half-life measured is 39.5hrs. Density plots shown above data points are forward-scatter (horizontal axis) vs. propidium iodide live/dead intensity (vertical axis). TNFα did not induce significant cell death over 72hrs in LTR-GFP-Tat or LTR-GFP controls (Supplementary Information).

Figure 4: SirT1 over-expression decreases positive-feedback strength and increases the probability of latency. **a.** Noise ACF for full-length HIV-1 (blue) and SirT1 over-expression in full-length HIV-1 (red). Over-expressing SirT1 yields weaker positive-feedback strength compared to full-length HIV-1 alone ($\tau_{1/2}$ = 1.35±0.08 vs. $\tau_{1/2}$ = 1.76±0.08, respectively). **b.** Analytical flow cytometry data (% GFP off cells from triplicate sorts) collected 96hrs post FACS sorting of TNFα activated populations of SirT1 over-expressing (red) and full-length HIV-1 (blue) sorts. SirT1 over-expressing cells exhibit significantly higher probability of entering latency after transactivation.