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HIV-1 positive feedback and lytic fate

Iftach Nachman & Sharad Ramanathan

The HIV viral lifecycle includes infection of a host cell, followed by a critical decision between latency and lysis. A new study suggests that positive feedback in the HIV-1 promoter, involving Tat protein and gene expression, has a role in this critical choice of fate.

The HIV viral lifecycle involves infection of a host cell, followed by reverse transcription and integration of the proviral DNA into the host genome. At that point, the proviral DNA can either remain latent[1] or activate transcription, leading to the creation of new viral particles and cell lysis. What factors regulate the decision to progress to cell lysis after HIV-1 infection? On page 466 of this issue, Weinberger *et al.*[2] show that positive feedback in the HIV-1 viral promoter serves as a tool for increasing viral production, potentially enhancing the probability of lytic events.

Critical switches

Several notable examples of switches regulating key developmental decisions have been studied. In the classic example of λ phage, a bistable switch controls the decision between lysis and lysogeny[3]. In *Bacillus subtilis*, cells are driven temporarily into a competent state through stochastic excitation of a fast positive feedback loop and fall out of it as a result of competing slower negative feedback[4]. In budding yeast, variable and gradual accumulation of a master transcription factor leads cells to enter sporulation at different times[5]. In all these examples, the stochastic nature of the underlying mechanism leads a population of identical cells to make different decisions. Weinberger *et al.*[2] now propose variability in transient pulses of viral gene expression as a mechanism for regulating the critical viral decision leading to latency versus lysis.

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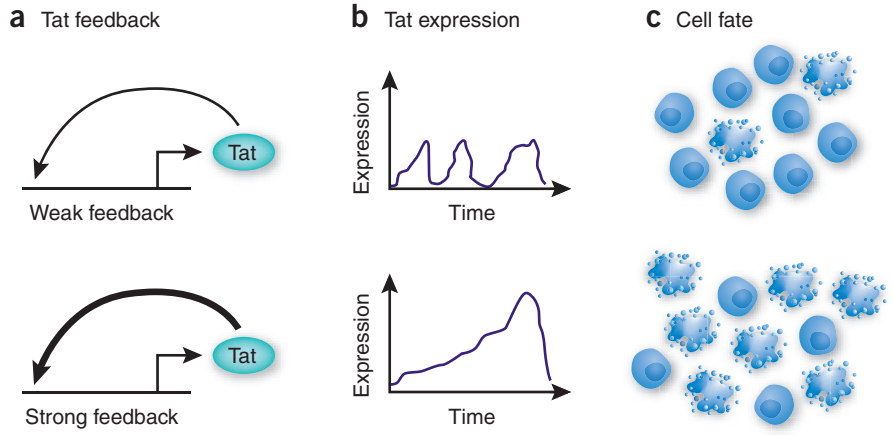

Figure 1 Relationship between feedback strength, Tat expression transients and fate decisions. (**a**) Tat transactivates the viral LTR promoter in a positive feedback loop. (**b**) A weaker level of feedback results in shorter expression transients (top). (**c**) As a result, fewer viral particles are created, and the probability of lysis is lower (top). In comparison, a stronger level of feedback (**a**, bottom) results in longer expression transients (**b**, bottom), more viral particles and a higher probability of lysis (**c**, bottom).

At the heart of this mechanism lies a viral protein named Tat (transactivator of transcription), which drives viral replication by activating RNA polymerase to enhance transcript elongation from the HIV-1 promoter. As Tat resides under the control of the same promoter, it enhances its own transcription via a positive feedback loop[6]. Weinberger and colleagues have previously shown that, depending on the site of integration, the viral promoter activity can show a stochastic 'bifurcating' pattern in a clonal population, where some of the cells have high promoter activity while others have low activity[7]. They later suggested that Tat protein and its activity are short-lived, leading to a 'dissipative' feedback circuit, where transient expression from the viral promoter decays to a single stable 'off' state[8]. Thus, the previously observed bimodality at a single time point could reflect cells asynchronously progressing through transient viral gene expression. If indeed the silent 'off' state is the only stable one, how is the lytic state achieved?

To address the question of how the Tat feedback circuit may affect cell lysis, Weinberger *et al.*[2] analyzed fluctuations from the Tat promoter and found that the positive feedback of Tat extended transient gene expression bursts (so-called 'transients') for sufficiently long periods, allowing for lysis (**Fig. 1**). The authors used autocorrelation analysis, a standard statistical method, to measure the typical length of time over

which fluctuations last. In theory, a blip in the signal (that is, some fluctuation about the mean steady state value) will continue longer with positive feedback than without, leading to a more slowly decaying autocorrelation function. The authors demonstrated this effect experimentally by weakening the positive feedback. Using either overexpression of a human inhibitor of Tat or introduction of a specific mutation in the *Tat* gene, they reduced the feedback strength and subsequently measured a decrease in the autocorrelation function, suggesting that the expression transients were shorter lived. They also found that reducing the viral feedback resulted in a higher latency rate by using a GFP reporter to measure promoter activity in cells. The human inhibitor that the authors overexpressed is a protein called SirT1 (a histone deacetylase and homolog of the yeast Sir2 protein), which de-acetylates and de-activates Tat, but which is also required for Tat transactivation[9]. SirT1 belongs to the sirtuin family of proteins implicated in a range of functions, from metabolism and aging to differentiation. Therefore, it is interesting to speculate whether and how those other functions might modulate Tat feedback.

Implications for HIV lifecycle

Weinberger *et al.* describe the Tat circuit as a generator of variable-length expression transients and propose that these variable transients serve as a probabilistic switch between latency and lysis[2]. It is not yet clear, however, to what extent this positive feedback circuit plays such a role *in vivo*. In addition to such feedback in the viral gene circuits, the state of the host cell is likely to have an important role in regulating this post–HIV infection fate decision[10]. For example, HIV preferentially infects active T cells, whereas quiescent T cells are not prone to infection, and, if infected, they do not promote viral replication. A 'resting' T cell, in which weak signals promote entry into G1 but not into subsequent stages of the cell cycle, can provide the conditions needed for infection that is not followed by replication, leading to latency[10]. In this manner, it is possible that the decision between latency or active replication and lysis could be determined solely by changes in external signals and the host cell state and that the Tat feedback characterized here might have a secondary role in executing this fate decision. In future studies, it will be important to consider the relevance of feedback within the HIV viral components in the context of the state of the host cell.

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Delivery codes for fly transgenics

Thomas C Tubon Jr & Jerry C-P Yin

Chromosomal position effects influence the transcription of exogenously introduced transgenes. A new study identifies molecular tools that exploit these properties to fine-tune transgenic gene expression through the use of site-specific integration and the gypsy insulator element.

As in real estate, location is (almost) everything in transgenic biology. The local chromatin structure that surrounds a randomly inserted transgene considerably affects the level and spatial pattern of expression. In *Drosophila*, the traditional solution to this inherent variability has been to generate and sort through many independent insertions in order to find ones that match the experimental need, as this can be done quickly and cheaply. However, unlike in real estate, there is now a systematic solution for this problem in *Drosophila*. On page 476 of this issue, Michele Markstein and colleagues[1] use information from two unrelated lines of work—site-specific recombination and position-effect variegation—to provide an elegant solution to transgenic biology's 'real estate' problem.

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Transgenesis in *Drosophila*

Reverse genetic approaches rely heavily on inducible gene expression systems to establish the relationship between a gene and its function. The current arsenal of transgenic techniques has facilitated the study of the effects of single gene manipulations in the context of a living organism. These techniques have paved the way for new discoveries in diverse disciplines ranging from learning and memory formation to disease pathogenesis. In *Drosophila*, several widely used approaches for transgenic gene manipulation are currently available. The first type involves placing the gene of interest under the control of the heat shock 70 (Hsp70) promoter, enabling a defined window of expression[2]. Hsp70-regulated transgenes are capable of rapid and robust induction, but they lack the ability to spatially restrict expression to specific tissues. A second approach, which uses the bipartite Gal4–upstream activating sequences (UAS) system[3], is the most widely used system in flies for achieving spatially restricted gene expression (**Fig. 1**). The primary limitation of this system is its inability to temporally control gene expression, a substantial problem whenever the function of a gene is being assayed late in development, usually after earlier usage. In light of this, variations of the Gal4-UAS system have been developed to introduce temporal control, including the use of the temperature-sensitive Gal80^{ts} protein[4,5], hormone ligands such as RU486 (GeneSwitch)[6–8] or the drug-based tetON/tetOFF system[9,10]. These developments allow temporal control to be added to the spatially restricted Gal4 system.

Although elegant, these approaches all rely on the use of one or more transgenes that are inserted randomly and that are therefore susceptible to the effects of local chromatin structure, whether repressive or activating, or acting with cell type–specific constraints. This randomness necessitates that multiple inserts be assayed, and even this is often not done thoroughly. For