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Stochastic gene expression as a molecular switch for viral latency

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Stochastic 'noise' arises from random thermal fluctuations in the concentration of protein, RNA, or other molecules within the cell and is an unavoidable aspect of life at the single-cell level. Evidence is accumulating that this biochemical noise crucially influences cellular auto-regulatory circuits and can 'flip' genetic switches to drive probabilistic fate decisions in bacteria, viruses, cancer, and stem cells. Here, we review how stochastic gene expression in key auto-regulatory proteins can control fate determination between latency and productive replication in both phage-λ and HIV-1. We highlight important new studies that synthetically manipulate auto-regulatory circuitry and noise, to bias HIV-1's ability to enter proviral latency. We argue that an appreciation of noise in gene expression may shed light on the mystery of animal virus latency and that strategies to manipulate noise may have impact on anti-viral therapeutics.

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Introduction

Consider a clonal cell population (i.e. genetically identical or isogenic cells) where all cells are divided from a single parent cell. The cells in the population will exhibit considerable cell-to-cell variation in the level of any specific gene product and this variation is referred to as the stochastic noise [1,2,3••]. The origin of this noise is biochemical: intracellular processes are driven by molecules randomly diffusing and colliding within the cell and are thus inherently stochastic. Specifically, noise in gene expression can arise from the random timing in individual reactions associated with promoter remodeling, transcription, and translation [4–6]. Moreover, intercellular differences in the *amount* of cellular components (for example, RNA polymerase, transcription factors, and ribosomes) also cause variations in expression levels. Measurements in live, single cells have shown that gene expression noise can lead to large statistical fluctuations in protein and mRNA levels in both prokaryotes and eukaryotes [7–10]. These fluctuations (i.e. noise) can have significant effects on biological function and phenotype.

On the one hand, noise can be problematic for essential proteins whose levels have to be tightly maintained within certain bounds for optimal performance [11] (e.g. noise can 'corrupt' information processing by cells [12]). On the other hand, noise could conceivably confer a benefit to the organism. Recent studies show that increasing noise in stress-related yeast proteins confers a selective advantage by allowing a larger fraction of the population to maintain stress-response protein levels above a threshold [2,13]. When confronted with two different pathways, cells may also exploit noise in key regulatory proteins for a probabilistic pathway selection and cell-fate determination [3••,14••]. Such stochastic fate decisions can create phenotypic heterogeneity across isogenic cell populations which can be beneficial in fluctuating environments [15•,16]. For example, during growth of a bacterial colony, a fraction of *E. coli* cells stochastically enter a persister state, a slow growing state that can elude the action of antibiotics [17]. While the persister strategy lowers the average colony growth rate under normal conditions, it pays off if *E. coli* randomly encounters antibiotics in its environment since the persister cells survive with high probability and resume normal growth after antibiotic treatment.

Like prokaryotes (and many other organisms), viruses also live in environments that change unpredictably. For viruses, fluctuating environments can be due to large variations in the number of susceptible host cells at any given time. In these fluctuating host populations, noise-driven *probabilistic* decisions (i.e. entry into latency or reactivation from latency) may provide a virus with a fitness advantage [18,19] similar to the advantage that noise confers in prokaryotes [20••,21,22]. In fact, a body of evidence now shows that noise can influence a fate decision between productive replication and dormancy (i.e. latency or lysogeny) in two viral systems: Human Immunodeficiency Virus type 1 (HIV-1) and bacteriophage-λ (phage-λ) [23,24].

Here, we compare and contrast the transcriptional regulatory mechanisms that phage-λ and HIV-1 use to exploit noise and control fate determination. In particular, we

review recent evidence demonstrating that HIV-1 proviral latency, like phage-λ lysogeny, is significantly influenced by stochastic gene expression, and that HIV-1 proviral latency is thus an inherent property of the viral life cycle [24,25••]. Importantly, we highlight recent results showing that altering HIV-1 auto-regulatory circuitry alters noise in HIV-1 gene expression and biases the HIV-1 fate decision for productive replication versus latency [26••].

Stochastic fate determination in phage-λ

Since the 1950s it has been known that genetically identical phage-infected bacteria, grown in the same environment, can undergo a 'developmental bifurcation' where one fraction of infected cells enter a lytic replication state and other infected cells enter a lysogenic state where phage-λ remains dormant [27]. In the late 1990s Arkin *et al.* developed computational models for the phage-λ gene network that argued that stochastic noise in gene expression together with an underlying 'bistability' in phage-λ's gene network, could account for the lysis–lysogeny developmental bifurcation [23]. The phage-λ viral gene circuit consists of a double negative feedback loop, where proteins CI and Cro mutually repress each other's expression. This feedback creates a bi-stable epigenetic switch where protein levels can lock into two different states: high Cro and low CI (which corresponds to lysis) or low Cro and high CI (which corresponds to lysogeny) [28]. Random timing in individual biochemical reactions during the early phase of infection can create small intercellular differences in the concentration of these repressor proteins. These differences are significantly amplified by the Cro-CI mutual repression causing an initial homogenous cell population to bifurcate into two subpopulations that have different developmental outcomes [23]. Similar stochastic models have also been proposed to explain why only a fraction of lysogens become lytic when induced with ultraviolet (UV) irradiation [29].

Importantly, phage-λ encodes a negative feedback mechanism where CI represses its own transcription [28]. This negative feedback may stabilize the lysogenic state against stochastic fluctuations. Such negative auto-regulatory loops are common gene network motifs within cells that suppress noise in protein levels [30]. Tight regulation of CI ensures that CI levels do not become small by random chance, which may cause a spontaneous transition to the lytic state. Negative feedback also prevents CI levels from becoming large, and high CI levels may desensitize the lysogenic state to induction signals [31].

Deterministic factors such as cell volume and MOI can bias probabilistic fate decisions

In the early 1970s, Phillippe Kourilsky performed a set of experiments that demonstrated how the frequency of lysogeny was dependent on the number of input phage-λ particles per cell (i.e. the multiplicity-of-infection or MOI) and that increased MOI correlated with increased lysogeny frequency [32]. Later experiments by Ira Herskowitz [33] showed that nutritional state of the cell also heavily biased lysogeny. These experimental correlations between MOI (or nutritional state) and lysogeny were in fact partly the inspiration for Arkin's stochastic lysis–lysogeny model and this stochastic model generates variable fractions of lytic and lysogenic cells at different MOIs, nutritional states, and even different cell volumes [23]. In fact, high MOI and small cell volume have a striking equivalence: if many phage infect a large cell, or if a single phage infects a much smaller cell, in both cases the initial concentration of lysogeny factors can be very high. A fascinating recent study [34••] demonstrates that cell size (like MOI) can indeed be a strong predictor of phage-λ infection outcome and that infections of smaller cells increase the probability of lysogeny, while infections of larger cells are biased toward lysis. Most strikingly, this study shows that even for the very largest and very smallest cells a fraction of cells consistently choose an outcome that is opposite to what would be predicted by cell volume alone. For example, some percentage of large cells become lysogens when the bacterial size dictates that they should become lytic. The fact that a percentage of cells enter the 'wrong' fate, strongly implies that phage-λ's fate decision is probabilistic. Dramatically, the authors capture this probabilistic decision making by direct single-cell observation of two daughter cells (of the same size) that take opposite developmental outcomes: one daughter cell becomes lytic, the other daughter develops into a lysogen. A deterministic model where cell size dictates the fate outcome cannot explain this striking observation. In fact, the lysis–lysogeny decision is apparently probabilistic even in the face of a strong deterministic pressure such as cell size. The probabilistic/stochastic model where the fate decision is 'biased' or nudged in one direction by deterministic pressures such as bacterial cell volume appears to be the most parsimonious with the available data.

Noise-induced variability in lysis times: implications for non-latent viruses

Apart from its role in decision-making, another important consequence of noise is that it can create significant variability in lysis times. This variability was recently quantified in phage-λ by monitoring the gene expression at different stages of the lytic cascade after induction with UV irradiation [35•]. The time taken to complete different lytic stages was found to be uncorrelated across cells. This result suggests that variation in lysis times arises due to random fluctuations in cascade components and not because the lytic cascade proceeds at different rates in different cells [35•]. Randomness in the time taken to complete the lytic phase can cause variability in the viral burst size (the number of progeny virus released per host

cell), which has been well documented in phage-λ [36]. Presumably, randomness in lysis times and burst size due to stochasticity in viral molecular components is a general feature across many viruses.

Stochastic fate determination in HIV-1

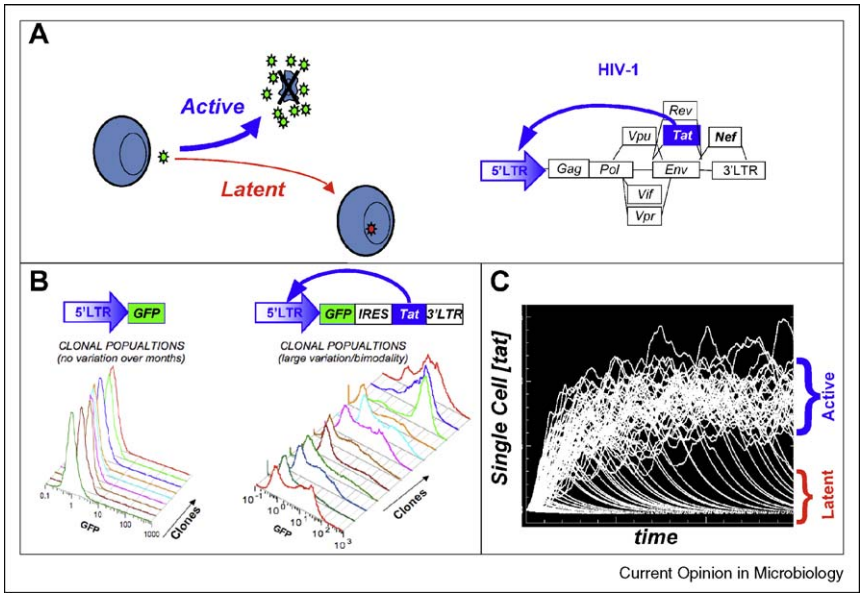
Like phage-λ, the Human Immunodeficiency Virus type 1 (HIV-1) can also enter one of two developmental fates: upon infecting a $CD4^+$ T lymphocyte HIV-1 can either enter an active replication state (productive infection) or enter a post-integration/proviral latent state (an analog of phage lysogeny). A substantial body of evidence, amassed by Siliciano and colleagues, has confirmed that HIV-1 proviral latent cells are quiescent for viral production and that viral gene expression is shut off during viral latency [37,38,39•,41]. These proviral latent cells are considered the most significant obstacle thwarting HIV-1 eradication from a patient [39•,40••] since latent cells can 'reactivate' during interruption of highly active anti-retroviral therapy (HAART) to generate rapid viral rebounds that re-establish pre-treatment HIV-1 levels [41].

Stochastic noise in the HIV-1 Tat auto-regulatory loop is sufficient to drive latency

While many host factors have been implicated in controlling HIV-1 replication and latency [42–44], there has been no conclusive identification of host cellular factors that direct an infected T lymphocyte to become latent. Importantly, the HIV-1 Tat protein (Trans-Activator of Transcription) is absolutely essential for active replication and latent reactivation [42,45,46]. 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 [39•,42,47]. 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 upregulating the expression of HIV Rev (the essential viral mRNA export factor) [48]. Our group and others have shown that stochastic fluctuations in Tat gene expression can act as a molecular switch and allow HIV-1 to enter a transcriptionally dormant state [24,49•].

To study noise in Tat expression, subgenomic HIV-1-based lentiviral vectors encoding only Tat and the green fluorescent protein (GFP) as a reporter were used to infect Jurkat T cells and construct isogenic (clonal) populations [24]. Strikingly, single cell level analysis of these isogenic populations by flow cytometry (Figure 1b), showed significant phenotypic bifurcation in isogenic Tat positive feedback populations despite all cells being genetically identical and grown from a single infected parent cell. The phenotypic bifurcation in isogenic Tat positive feedback populations was evidenced as one fraction of the infected cells exhibiting very high gene expression levels (on) and another fraction of infected cells exhibiting no viral gene expression (off). Importantly, isogenic populations

Figure 1



A minimal HIV Tat positive feedback circuit is *sufficient* to generate a 'latency' decision in the presence of noise. **(a)** The HIV-1 'proviral' genome with Tat positive feedback displayed and the HIV-1 developmental bifurcation between active replication and proviral latency after infection of a CD4+ T lymphocyte. **(b)** Jurkat T cells infected with either a control LTR-GFP or feedback LTR-GFP-IRES-Tat lentiviral vector, were single-cell sorted by FACS, grown into clonal populations and subsequently analyzed by flow cytometry. **(c)** Single-cell Tat trajectories of an HIV-1 gene-circuit model. Each trajectory represents the level of Tat within a single cell over time. Significant fluctuations in Tat levels exist and can drive a phenotypic bifurcation between two states.

infected with control lentiviral vectors lacking functional Tat positive feedback did not display this phenotypic bifurcation. Subsequent analysis showed that stochastic fluctuations in Tat gene expression were of primary importance in influencing this developmental bifurcation (or decision) in the HIV Tat positive feedback circuit.

The result that stochastic Tat gene expression could drive latency, coupled with important work by Eric Verdin's group [42] to develop state-of-the-art and accepted cell-culture models of HIV-1 latency, highlighted the fact that proviral latency was not a consequence of HAART or due to complex T cell maturation phenomena that occur only *in vivo*. The result that minimal Tat circuits can reproduce latency has the important implication that proviral latency is an inherent property of the HIV-1 lifecycle and is due to fundamental biochemical processes underlying the virus's regulatory network. Since proviral latency appears to be an inherent property of HIV-1 gene expression that has been conserved, it is likely that latency plays a role in the natural history of HIV-1 replication and probably provides the virus with a fitness advantage in the natural setting.

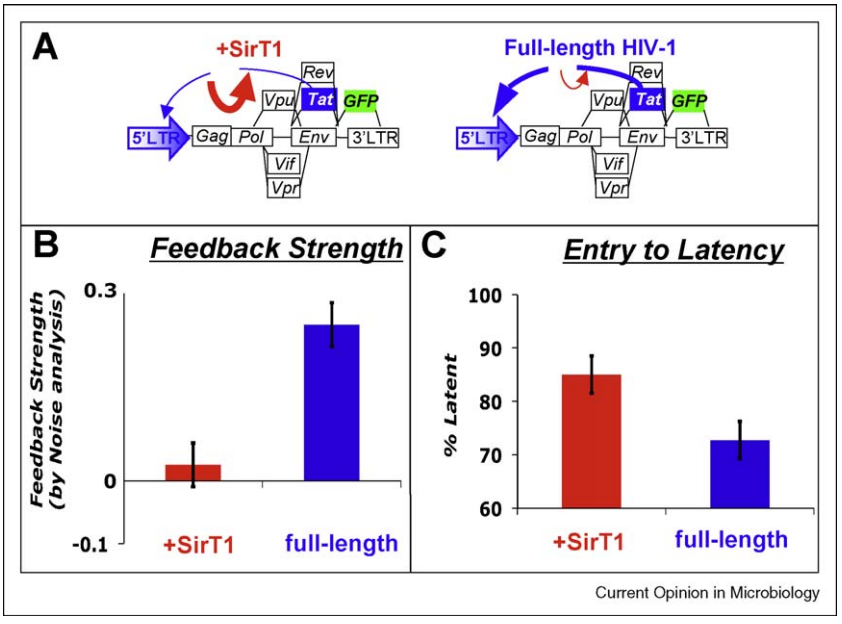
Unlike phage-λ, the HIV-1 Tat auto-regulatory loop lacks bistability

Based on the established bistability model in phage-λ [23], it seemed that phenotypic bifurcation in minimal HIV Tat circuits might be a result of biochemical noise driving Tat levels stochastically above or below a bistable threshold in HIV Tat. Molecularly, bistability requires some form of self-cooperative threshold, such as protein homo-multimerization or cooperative binding. The presence of such as threshold is typically measured by calculating a 'Hill' coefficient from a dose-response curve, as is done in the study of enzyme kinetics. A Hill coefficient >1 implies the presence of a self-cooperative threshold, whereas a Hill coefficient ≤1 rules out the presence of a self-cooperative threshold and necessarily rules out bistability. The Hill coefficient for the HIV Tat circuit was measured using an array of quantitative single-cell imaging methods and found to equal one [25••], thereby ruling out bistability in the HIV Tat circuit. Instead, it appears that HIV utilizes a different mechanism, *excitable* circuitry, to decide between alternate fates. In the excitable circuit model, cell fate is determined by the duration of transient Tat expression pulses (Figure 1c) and Tat mediated positive feedback acts to extend the lifetime of the these pulses. More specifically, short-lived Tat pulses allow for latency and long-lived Tat pulses drive active viral replication and reactivation from latency.

Weakening feedback strength alters noise and reduces entry into HIV-1 latency

The excitable circuit model presented a clear avenue for altering the HIV fate decision in single cells. In particular, if the Tat positive feedback strength could be diminished, then the duration of the Tat pulses would be shortened and infected cells would be more likely to

Figure 2



Weakening HIV-1 Tat positive feedback biases HIV-1 toward latency and limits reactivation. **(a)** Schematics of full length HIV-1 with *nef* replaced by a *gfp reporter* (blue) and the SirT1 overexpression effect (red). **(b)** SirT1 overexpression deacetylase Tat and reduces positive-feedback strength (red) as evidenced from gene expression noise analysis. **(c)** SirT1 overexpressing cells have a higher probability of entering latency and show decreased latent reactivation [26••].

enter latency. To test this excitable circuit model, a concept was borrowed from electrical circuit analysis: gene expression noise was exploited as an experimental probe. Specifically, by measuring the duration of stochastic fluctuations in Tat, feedback strength can be directly quantified through noise autocorrelation analysis [50]. This gene-expression noise autocorrelation method was used to determine Tat positive feedback strength in minimal and full length HIV-1 virus at the single-cell level [26••]. Most importantly, in the presence of a known down regulator of Tat function, the histone deacetylase SirT1 (Figure 2a) [51], feedback strength was significantly diminished (Figure 2a,b). Strikingly, cells with reduced feedback strength showed a significant shortening in the duration of Tat expression transients and an increase in the proportion of cells entering latency in this system (Figure 2c) [26••]. Thus, by manipulating feedback strength and the accompanying noise frequency profile, the HIV-1 latency decision can be altered, potentially for therapeutic benefit, as previously proposed [52].

Conclusion

Deterministic models are clearly insufficient to explain the probabilistic nature of phage-λ lysogeny and HIV-1 proviral latency. Conclusively stating that a biological process is stochastic is a strong statement and requires significantly more evidence than simply observing a distribution. However, we believe that extensive single-cell evidence now exists for the stochastic nature of a variety of biological fate-determination processes, for comprehensive reviews see [3••,14••], and specifically for viral latency decisions. One set of experiments that lends strong support to the stochastic model are noise perturbation experiments that eliminate or diminish the stochastic noise in a specific process and test whether the process retains a probabilistic phenotype. Recent studies in a number of systems [20••,21] have succeeded in specifically manipulating the noise in gene expression, without manipulating the deterministic mean in expression levels, and demonstrated that fate outcome is significantly altered. For HIV-1, these noise manipulation experiments provide strong evidence that proviral latency is significantly influenced by underlying stochastic noise in gene expression [26••]. Similar noise manipulation experiments in phage-λ would lend strong support to the stochastic model of lysis–lysogeny in phage-λ.

From the available evidence, viruses appear to exploit noise in gene expression by encoding auto-regulatory positive feedback gene circuits (or double negative feedback gene circuits) that may amplify noise to generate probabilistic 'molecular switches' for latency. If this proves correct, stochastic noise in gene expression noise may represent a novel therapy target to manipulate viral fate and this has been demonstrated in principle by manipulating HIV-1's Tat positive feedback circuit to bias the virus's ability to enter latency and reactivate [26••]. An understanding of noise in gene expression may also shed light upon latency in other viruses such as herpesviruses where latency is an integral of part of the viral life cycle. Herpesvirus reactivation from latency exhibits a probabilistic phenotype, which is evocative of a role for noise in gene expression. Stochastic gene expression from latent herpesvirus genomes has been reported in a beta herpesvirus [53] and recently hypothesized as a mechanism for reactivation from latency in an alpha herpesvirus [54•]. A system-level understanding of viral gene-network architecture, and its associated molecular noise, will probably be crucial to unravel the mystery of animal viral latency.

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