Preprints of the 19th World Congress
The International Federation of Automatic Control
Cape Town, South Africa. August 24-29, 2014

Stochastic analysis of protein-mediated and microRNA-mediated feedback circuits in HIV

Zachary Fox * **Abhyudai Singh** **

* *Department of Biomedical Engineering, University of Delaware, Newark, DE 19716 USA (e-mail: zfox@udel.edu)*
** *Department of Electrical and Computer Engineering, University of Delaware, Newark, DE 19716 USA (Tel: 302-831-8447; e-mail: absingh@udel.edu).*

Abstract: After infecting a $CD4^+$ T cell, Human Immunodeficiency Virus (HIV) can either replicate and kill the cell or enter latency, a dormant state of the virus where viral gene-expression is turned OFF. This cell fate decision between viral replication and latency is controlled by the viral regulatory protein, Tat. This protein is known to activate its own production, creating a positive feedback circuit. Our previous work has shown that a stochastic model of this feedback circuit exhibits bimodal distributions of Tat levels, even though this circuit lacks deterministic bistability. The modes of the distribution correspond to infected cells with high Tat levels (corresponding to viral replication) or no Tat at all (corresponding to HIV latency).

Experimental evidence points to an additional positive feedback loop mediated through a microRNA: a host microRNA targets Tat mRNA for degradation and Tat protein blocks synthesis of this microRNA. Here we investigate the interplay between Tat-mediated and microRNA-mediated positive feedback loops using deterministic and stochastic modeling. Our results show that these positive feedbacks together can exhibit deterministic bistability if the microRNA-mRNA interaction is sufficiently strong. Intriguingly, stochastic analysis reveals bimodal distributions for Tat even for parameter regimes where the coupled feedback system is not bistable. In summary, addition of the micro-mediated feedback loop can lead to bimodal Tat levels for wide a range of parameter values, and suggests a role for microRNAs in the viral cell fate decision *in vivo*.

Keywords: Gene Expression; Positive Feedback; microRNAs; Noise; HIV

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) is a raging epidemic across the world, with millions of people currently infected by the virus. HIV infects cells of the immune system, in particular, $CD4^+$ T cell lymphocytes. After infecting an individual cell, HIV typically enters an active replication state, which kills the infected cell producing viral progeny (Seth et al. [2005], Perelson et al. [1996]). However, in some infected cells HIV enters latency, a quiescent or dormant state of the virus where viral gene-expression is turned OFF (Chun et al. [1997]). While active replication destroys $CD4^+$T cells and leads to AIDS, HIV latency is considered the biggest obstacle preventing HIV eradication from a patient (Han et al. [2007], Richman et al. [2009]). In particular, latently infected cells are long-lived drug-resistant viral reservoirs and allow reemergence of HIV to pre-treatment levels once anti-retroviral drug therapy is discontinued (Richman et al. [2009]).

Tat, a HIV regulatory protein expressed immediately after infection, is essential for viral replication. Tat activates transcription of its own promoter creating a positive feedback circuit, which critically influences the viral cell fate decision between viral replication and latency (Weinberger and Shenk [2006], Pearson et al. [2008]). Experimental data shows that stochastic expression of Tat protein coupled with the positive feedback loop generates bimodal distributions of Tat levels, where individual cells either have high levels of Tat (corresponding to viral replication) or no Tat at all (corresponding to HIV latency) (Singh and Weinberger [2009]). Further work revealed that the Tat feedback circuit lacks deterministic bistability (i.e. the existence of two stable fixed points) (Weinberger and Shenk [2006], Weinberger et al. [2008]). A key question to be addressed is: how does bimodality arise in a monostable genetic positive feedback circuit?

Detailed analysis has shown that bimodality is possible in a stochastic model of the Tat positive feedback loop (Singh [2012]). However, the parameter space where bimodality arises is quite restrictive (Singh [2012]). Recent experiments have shown the existence of an additional positive feedback loop mediated through a microRNA: a host microRNA targets Tat mRNA for degradation and Tat protein blocks synthesis of this microRNA (Triboulet

et al. [2007], Corbeau [2008]). Here we investigate how the addition of the microRNA-mediated positive feedback loop affects Tat protein level distribution across a population of infected cells. Our analysis reveals that a strong microRNA-mRNA interaction can result in deterministic bistability, where Tat levels can stably exist at high and low levels. Moreover, stochastic simulations show that bimodal Tat levels arise for a wide range of parameter values, including values when the coupled feedback system lacks bistability.

The paper is organized as follows. Results from deterministic and stochastic modeling of the Tat positive feedback loop are presented in Section 2. In section 3, a deterministic model combining Tat-mediated and microRNA-mediated feedback loops is developed and analyzed. Stochastic simulations of the coupled feedback system are also presented in Section 3. Finally, conclusions and future work are discussed in Section 4.

2. MODELING TAT POSITIVE FEEDBACK CIRCUIT

Auto-regulatory positive feedback loops are common motifs in gene networks (Alon [2006]). One such positive feedback loop is found in HIV: Tat protein expressed from the HIV promoter activates transcription from its own promoter (Figure 1).

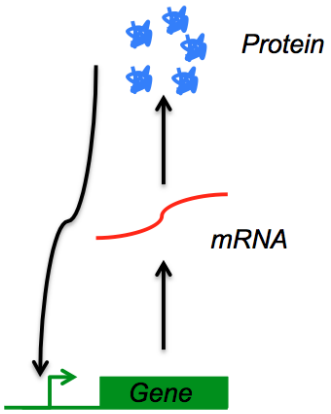

Fig. 1. Simple positive genetic feedback circuit, where a protein expressed from a gene up-regulates the transcription of its own mRNA.

2.1 Deterministic modeling of the Tat feedback circuit

Let $p(t)$ and $m(t)$ denote the Tat protein level and the Tat mRNA level at time t, respectively. Then, the time evolution of $p(t)$ and $m(t)$ can be modeled deterministically through the following set of ordinary differential equations (ODEs):

$$\frac{dm}{dt} = f_1(p(t)) - \gamma_m m(t) \tag{1a}$$

$$\frac{dp}{dt} = k_p m(t) - \gamma_p p(t). \tag{1b}$$

We denote by γ_p, γ_m and k_p the protein degradation rate, mRNA degradation rate and mRNA translation rate, respectively. Positive feedback is incorporated by assuming that the mRNA transcription rate

$$f_1(p) = \frac{k_m(b + (c_1 p)^h)}{1 + (c_1 p)^h} \tag{2}$$

is a monotonically increasing function of the protein level. Here, k_m is the maximum transcription rate, c_1 is the strength of the positive feedback and h is the Hill coefficient, which determines how sigmoidal is the mRNA transcription rate as function of the protein level. When $p = 0$, transcription occurs at a basal rate of $k_m b$, where $0 < b < 1$. In this system, $b \ll 1$ because transcription from the HIV promoter is known to be defective in the absence of Tat: RNA polymerase II stalls 50-70 nucleotides after initiating transcription from the HIV promoter (Kao et al. [1987]). These stalled polymerases abort transcription with a high probability, making basal transcription from the HIV promoter very weak. However, a few RNAPII are still able to transcribe a full-length mRNA, which go on to make the Tat protein. Tat directly binds to the stalled RNAPII-RNA complex and hyperphosphorylates RNAP II. This post-translation modification enhances RNAPII transcriptional processivity and allows it to complete the transcription process creating an efficient feedback loop through Tat (Karn [1999], Stevens et al. [2006]).

Steady-state protein level $\bar{p}$ is determined by the solution of the following equation

$$\frac{k_p f(\bar{p})}{\gamma_m} = \gamma_p \bar{p}. \tag{3}$$

High or low Hill coefficients can effect the stability of the system. When $h = 1$, the left-hand-side of (3) increases linearly with $\bar{p}$ and saturates. This leads to a single stable fixed point and the system is deterministically monostable. However, when the Hill coefficient is high, the left-hand-side of (3) increases sigmoidally with $\bar{p}$ and can result in two stable equilibrium protein levels (i.e., bistability).

A bistable Tat feedback circuit could potentially explain the two different fates of the infected cell, as the circuit is locked in either a low or a high Tat level state. The former state would correspond to HIV latency, and high Tat levels leading to viral replication. However, experimental measurements of h in the HIV system have shown that the Tat feedback circuit has a Hill coefficient equal to one (Weinberger and Shenk [2006]). Hence, deterministic bistability cannot be used as a mechanism to explain the existence of distinct high and low Tat level states of the positive feedback circuit. In the remainder of this paper we assume $h = 1$ in (2).

2.2 Stochastic modeling of the Tat feedback circuit

The environment within living cells is incredibly noisy with biochemical species randomly bumping and reacting with each other. This inherent probabilistic nature along with low population counts of cellular species creates considerable stochastic fluctuations in protein copy numbers over time inside individual cells. Under such conditions, a differential equation model no longer adequately captures the system dynamics. Gene networks are typically analyzed through stochastic models (Cinquemani et al. [2008], Singh [2011], Singh and Soltani [2013]) and these modes

can exhibit behaviors such as noise-induced oscillations (Bratsun et al. [2005]), stochastic focusing (Paulsson et al. [2000]) and stochastic resonance, (Wanga et al. [2007]) that cannot be realized in deterministic models.

We begin by providing a brief overview of stochastic modeling of genetic circuits. First transcription, translation and mRNA/protein degradation are represented by the following set of reactions:

$$gene_1 \xrightarrow{f_1(p)} gene_1 + mRNA \tag{4a}$$

$$mRNA \xrightarrow{k_p} mRNA + protein \tag{4b}$$

$$mRNA \xrightarrow{\gamma_m} \phi; \quad protein \xrightarrow{\gamma_p} \phi. \tag{4c}$$

For the transcription reaction (4a), rate $f_1(p)$ is the same as in (2). As before, protein and mRNA levels in an individual cell at time t are denoted $p(t)$ and $m(t)$, but are now integer-valued stochastic processes. Stochastic modeling of the feedback circuit corresponds to a stochastic formulation of these reactions, where each reaction is a probabilistic event and fires at random times (Gillespie [2001]). More specifically, the probability that the reactions (4a)-(4c) occur in the infinitesimal time interval $(t, t+dt)$ is given by $f_1(p)dt$, $k_p m(t)dt$, $\gamma_m m(t)dt$, and $\gamma_p p(t)dt$, respectively. Whenever a particular reaction occurs, $m(t)$ and $p(t)$ change by integer amounts based on the stoichiometry of the reaction. For example, protein count increases by one whenever a translation reaction (4b) occurs.

Previously, we considered a special case $\gamma_p \ll \gamma_m$ (i.e., the mRNA degrades much faster than the protein) (Singh [2012]). In this limit, (4) reduces to the following system of reactions

$$gene_1 \xrightarrow{f_1(p)} gene_1 + B \times protein \tag{5a}$$

$$protein \xrightarrow{\gamma_p} \phi, \tag{5b}$$

where a gene makes protein directly in bursts B, and B is a geometrically distributed random variable with mean $1/\alpha = k_p/\gamma_m$ (Shahrezaei and Swain [2008], Singh and Hespanha [2009]). A detailed stochastic analysis revealed that this reduced system can exhibit a bimodal distribution for protein levels in spite of the fact that the system is deterministically monostable (Singh [2012]). In particular, if b is sufficiently small (*i.e.* rate of transcription is low in the absence of the protein) such that

$$\gamma_p < k_m < \gamma_p/b \tag{6}$$

and the positive feedback strength is sufficiently strong $c_1 > c^*$, where c^* is given by:

$$c^* = \frac{a_2 + \sqrt{a_2^2 - 4\alpha^2\left(\frac{k_m}{\gamma_p} - 1\right)^2}}{2\left(\frac{k_m}{\gamma_p} - 1\right)^2} \tag{7a}$$

$$a_2 = 2\alpha\left(\frac{k_m}{\gamma_p} - 1\right) + 4\alpha\left(1 - \frac{k_m b}{\gamma_p}\right), \tag{7b}$$

then the steady-state protein count distribution is bimodal (Singh [2012]). Although these analytical results are restricted to the case $\gamma_p \ll \gamma_m$, stochastic simulations of the set of reactions (4) show (6) and (7) are good indicators of bimodality for all values of γ_m and γ_p (Figure 2).

To understand why bimodality arises note that when $b = 0$, $f_1(0) = 0$ and no protein synthesis occurs in the absence of the protein. Once the protein level hits zero by random chance, recovery is impossible and $p(t) = 0$ with probability one for all future time (i.e., stochastic extinction). A bimodal protein distribution results for low non-zero values of b (Figure 2; middle plot), where one of the modes represents high Tat levels corresponding to the monostable state of the ODE model. The other mode corresponds to no Tat protein, as the system is stuck here for long times due to inefficient transcription in the absence of Tat.

The Tat feedback system has all the hallmarks for noise-induced bimodality: dysfunction transcription in the absence of Tat (low b) and a strong positive feedback loop (high c_1). Although these results are encouraging, the parameter regime were bimodality arises is very restrictive: low values of b and c_1 values slightly higher than c^*. The steady-state probability of $p = 0$ decreases dramatically with increasing c_1, and for c_1 values significantly higher than c^*, the protein distribution is effectively unimodal (Figure 2; right-most plot). Below we investigate how these results are affected by the addition of a micro-RNA mediated positive feedback loop.

3. DETERMINISTIC MODELING OF MICRORNA-MEDIATED FEEDBACK LOOP

microRNAs are small non-coding RNA molecules that regulate gene-expression in a variety of regulatory networks (Siciliano et al. [2013]). They typically bind to mRNAs, targeting them for degradation or preventing the mRNA from being translated. Recent work provides evidence of both host microRNAs targeting viral gene-expression, and microRNAs expressed from the HIV genome altering specific cellular functions for viral replication. Here, we consider the case where a host microRNA targets Tat mRNA for degradation. Tat blocks the production of this microRNA by inhibiting the microRNA biogenesis machinery, therefore creating a second positive feedback loop as shown in Figure 3.

3.1 Model formulation

Coupled Tat-mediated and microRNA-mediated feedbacks are modeled through the following set of ODEs:

$$\frac{dm}{dt} = f_1(p(t)) - \gamma_m m(t) - kr(t)m(t) \tag{8a}$$

$$\frac{dr}{dt} = f_2(p(t)) - \gamma_{micro} r(t) - kr(t)m(t) \tag{8b}$$

$$\frac{dp}{dt} = k_p m(t) - \gamma_p p(t) \tag{8c}$$

where $m(t)$, $r(t)$ and $p(t)$ denote mRNA, microRNA and protein levels at time t, respectively. The term $kr(t)m(t)$ is (8a) and (8b) represents loss of $m(t)$ and $r(t)$ via the reaction

$$mRNA + microRNA \xrightarrow{k} \phi \tag{9}$$

where the microRNAs bind to mRNAs to form a complex that is degraded instantaneously. Rate k is referred to as

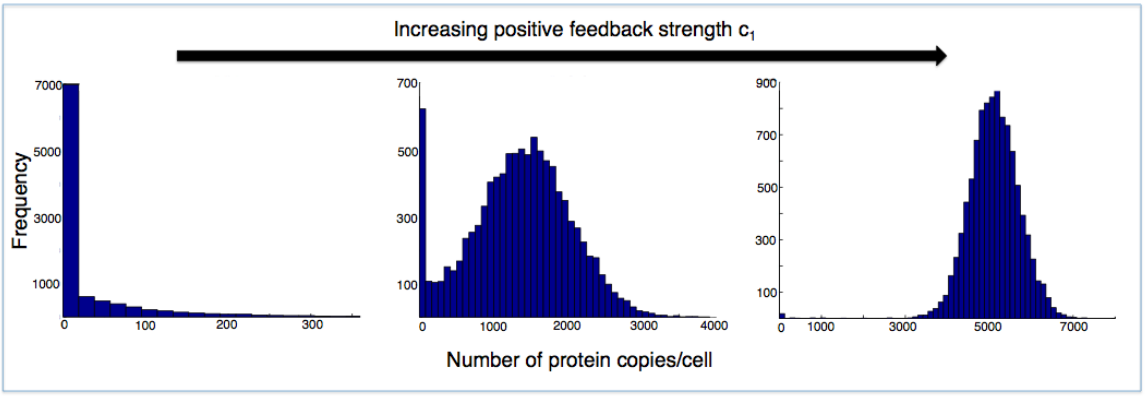

Fig. 2. Distribution of Tat protein in a stochastic model of the Tat feedback circuit. Distributions were obtained by stochastic simulation of (4) using software StochKit2 (Sanft et al. [2011]), which is based on the Stochastic Simulation Algorithm (Gillespie [2001]). Parameters were taken as $k_p = 80$, $\gamma_p = 1$, $k_m = 100$, $\gamma_m = 1$, $b = 0.001$. For these values $c^* = 0.000153$ from (7). The positive feedback strength was taken as $c_1 = 0.00010$, $c_1 = 0.000165$, and $c_1 = 0.00032$ from left to right. For $c_1 < c^*$ the distribution is unimodal with a zero model (left). Bimodality arises in the middle plot for c_1 values slightly great than c^*, however for higher values of c_1 the distribution is effectively unimodal (right plot). Note that (6) and (7) are good indicators of bimodality here in spite of the fact that $\gamma_m = \gamma_p$.

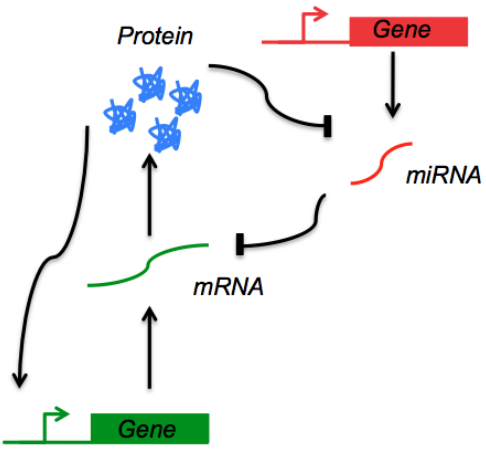

Fig. 3. Schematic of the Tat-mediated and microRNA-mediated positive feedback loops. A positive feedback is generated when Tat inhibits production of a host microRNA, and the microRNA blocks Tat synthesis by degrading its mRNA (Triboulet et al. [2007], Corbeau [2008]). Arrows represent activation, and bars at end of the line denote inhibition.

the mRNA-microRNA interaction strength and microRNAs are assumed to decay at a constant rate γ_{micro}. Affect of Tat on the microRNA is captured through the microRNA production rate

$$f_2(p) = \frac{k_{micro}}{1 + c_2 p(t)} \tag{10}$$

which monotonically decreases with increasing $p(t)$. Here k_{micro} is the maximum microRNA production rate and c_2 can be interpreted as the strength of Tat inhibition. When $k = 0$ (i.e., no microRNA interaction), (8) reduces to the deterministically monostable system (1). Below, we investigate if the additional microRNA feedback could push the system towards bistability.

3.2 Bistability condition

For simplicity we assume $c = c_1 = c_2$, i.e., the Tat feedback strength is equal to the strength of Tat-mediated microRNA inhibition. Given inefficient Tat transcription in its absence, we further assume $b \approx 0$. Setting the left-hand-side of (8) to zero and solving for the steady-states reveals three fixed points. First is the trivial equilibrium

$$\bar{m} = 0, \bar{p} = 0, \bar{r} = \frac{k_{micro}}{\gamma_{micro}}. \tag{11}$$

The other two are

$$\begin{aligned} \bar{p} &= \frac{-\beta_1 \pm \sqrt{4ck\gamma_m \frac{k_p}{\gamma_p}\alpha_1 + \beta_1^2}}{2c\gamma_m k}, \\ \bar{m} &= \frac{\gamma_p \bar{p}}{k_p}, \qquad \bar{r} = \frac{f_2(\bar{p})}{\gamma_{micro} + k\bar{m}}, \end{aligned} \tag{12}$$

where

$$\beta_1 = \gamma_m k + \frac{ck_p}{\gamma_p}(\gamma_m \gamma_{micro} - k k_m), \tag{13a}$$

$$\alpha_1 = -\gamma_m \gamma_{micro} - k k_{micro} + \frac{c\gamma_{micro} k_m k_p}{\gamma_p}, \tag{13b}$$

and $\bar{m}$, $\bar{p}$, $\bar{r}$ denote steady-state levels of mRNA, Tat, and microRNA, respectively. Standard stability analysis of these fixed points reveals parameter regimes for monostability and bistability.

For sufficiently low Tat feedback strength $c < \frac{\gamma_m \gamma_p}{k_m k_p}$ the trivial equilibrium

$$\bar{m} = 0, \bar{p} = 0, \bar{r} = \frac{k_{micro}}{\gamma_{micro}} \tag{14}$$

with no Tat and high levels of microRNA is the only viable (non-negative values for $\bar{m}, \bar{p}, \bar{r}$) and stable fixed point. For sufficiently high Tat feedback strength $c > \frac{\gamma_m \gamma_p}{k_m k_p}$ and weak microRNA-mRNA interaction such that

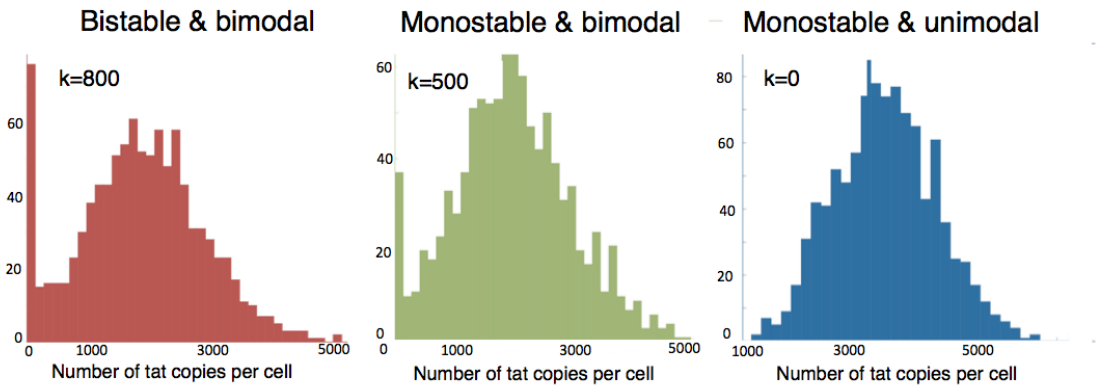

Fig. 4. Distribution of Tat protein in a stochastic model of the dual Tat-mediated and microRNA-mediated positive feedback loops. Parameter values taken as $k_p = 3200$, $k_m = 27$, $k_{micro} = 5$, $\gamma_m = 20$, $\gamma_p = 1$, $\gamma_{micro} = 50$, $b = .01$ and $c = 0.001$. For these values the critical interaction strength above which the system is bistable is $k > 664$. From left to right, $k = 800$, $k = 500$, and $k = 0$. Note that the Tat distribution is bimodal when the system in bistable (left). Moreover, is some cases bimodality also arises when the system in monostable (middle plot). Distributions were obtained by stochastic simulation of (19) on StochKit2. Parameter values were chosen such that is the absence of the microRNA ($k = 0$), Tat copy number distribution is unimodal with a non-zero mode.

$$k < \frac{\gamma_{micro}(\frac{ck_mk_p}{\gamma_p} - \gamma_m)}{k_{micro}}, \tag{15}$$

the trivial equilibrium (14) is unstable and

$$\begin{aligned} \bar{p} &= \frac{-\beta_1 + \sqrt{4ck\gamma_m \frac{k_p}{\gamma_p}\alpha_1 + \beta_1^2}}{2c\gamma_m k}, \\ \bar{m} &= \frac{\gamma_p \bar{p}}{k_p}, \qquad \bar{r} = \frac{f_2(\bar{p})}{\gamma_{micro} + k\bar{m}}, \end{aligned} \tag{16}$$

with high protein and low microRNA levels is the only viable and stable equilibrium. Finally for

$$c > \frac{\gamma_m\gamma_p}{k_mk_p}, \; k > \frac{\gamma_{micro}(\frac{ck_mk_p}{\gamma_p} - \gamma_m)}{k_{micro}} \tag{17}$$

the system is bistable with both the no-protein equilibrium (14) and the high-protein equilibrium (16) being stable fixed points and

$$\begin{aligned} \bar{p} &= \frac{-\beta_1 - \sqrt{4ck\gamma_m \frac{k_p}{\gamma_p}\alpha_1 + \beta_1^2}}{2c\gamma_m k}, \\ \bar{m} &= \frac{\gamma_p \bar{p}}{k_p}, \qquad \bar{r} = \frac{f_2(\bar{p})}{\gamma_{micro} + k\bar{m}}, \end{aligned} \tag{18}$$

being an unstable fixed point. In summary, for sufficiently high Tat feedback strength and strong microRNA-mRNA interaction (as determined by (17)), the system of dual positive feedbacks is bistable.

3.3 Stochastic Modeling with microRNA Feedback

While bistability is often a good indicator of bimodal distributions in the stochastic version of the model, bimodality in a stochastic model does not often imply deterministic bistability. To investigate distributions of Tat levels across a cell population we perform stochastic simulation of the coupled feedback system. Model (8) can be represented by the following set of reactions:

$$gene_1 \xrightarrow{f_1(p)} gene_1 + mRNA \tag{19a}$$

$$gene_2 \xrightarrow{f_2(p)} gene_2 + microRNA \tag{19b}$$

$$mRNA \xrightarrow{k_p} mRNA + protein \tag{19c}$$

$$mRNA + microRNA \xrightarrow{k} \phi \tag{19d}$$

$$mRNA \xrightarrow{\gamma_m} \phi; \;\; microRNA \xrightarrow{\gamma_r} \phi \tag{19e}$$

$$protein \xrightarrow{\gamma_p} \phi \tag{19f}$$

which are analyzed using the stochastic simulation package StochKit2 (Sanft et al. [2011]). As expected, when the microRNA-mRNA interaction strength k is sufficiently high such that the system is bistable (as determined by (17)), Tat distributions are bimodal (left plot in Figure 4). Intriguingly, bimodal distributions are observed for moderate values of k for which the system is deterministically monostable (middle plot in Figure 4). Thus, bimodality is observed not only for parameter values corresponding to bistability, but also much beyond it.

4. CONCLUSIONS

A system of dual positive feedback circuits mediated by the HIV Tat protein and a host microRNA were modeled (Figure 3). Experimental data shows bimodal Tat levels across a population of infected cells (Weinberger et al. [2005]). We investigated parameter regimes where bimodality, i.e., some cells have high Tat levels and others have no Tat, is feasible. Although the Tat feedback loop is itself monostable, deterministic modeling revealed that the dual feedback system is bistable if the Tat positive feedback strength and the mRNA-microRNA interaction strength are sufficiently strong. Stochastic modeling showed bimodal distributions for Tat levels for parameter regimes where the coupled feedback system is bistable, and also for cases where the system lacks bistability (Figure 4). In summary, addition of the micro-mediated feedback loop enhances the region of parameter space where Tat levels are bimodal. Our results suggest that microRNA control of

HIV gene expression may be critical in stabilizing Tat levels at high and low levels in different infected cells, which ultimately leads to different viral infection outcomes.

ACKNOWLEDGEMENTS

AS is supported by the National Science Foundation Grant DMS-1312926, University of Delaware Research Foundation (UDRF) and Oak Ridge Associated Universities (ORAU). ZF was supported by Howard Hughes Medical Institute.

REFERENCES

Uri Alon. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman and Hall/CRC Mathematical and Computational Biology, 2006.

Dmitri Bratsun, Dmitri Volfson, Lev S. Tsimring, and Jeff Hasty. Delay-induced stochastic oscillations in gene regulation. *Proceedings of the National Academy of Sciences*, 102:14593–14598, 2005.

Tae-Wook Chun, Lieven Stuyver, Stephanie B. Mizell, Linda A. Ehler, Jo Ann M. Mican, Michael Baseler, Alun L. Lloyd, Martin A. Nowak, and Anthony S. Fauci. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proceedings of the National Academy of Sciences*, 24:13193–13197, 1997.

Eugenio Cinquemani, Andreas Milias-Argeitis, Sean Summers, and John Lygeros. Stochastic dynamics of genetic networks: modelling and parameter identification. *Bioinformatics*, 24:2748–2754, 2008.

Pierre Corbeau. Interfering RNA and HIV: Reciprocal Interferences. *PLoS Pathog*, 4:e1000162, 2008.

Daniel T. Gillespie. Approximate accelerated stochastic simulation of chemically reacting systems. *J. of Chemical Physics*, 115:1716–1733, 2001.

Yefei Han, Megan Wind-Rotolo, Hung-Chih Yang, Janet D. Siliciano, and Robert F. Siliciano. Experimental approaches to the study of HIV-1 latency. *Nature Reviews Microbiology*, 5:95–106, 2007.

Shaw-Yi Kao, Andrew F. Calman, Paul A. Luciw, and B. Matija Peterlin. Anti-termination of transcription within the long terminal repeat of HIV-1 by Tat gene product. *Nature*, 330:489–493, 1987.

Jonathan Karn. Tackling Tat. *Journal of Molecular Biology*, 293:235–254, 1999.

Johan Paulsson, Otto G. Berg, and Mans Ehrenberg. Stochastic focusing: Fluctuation-enhanced sensitivity of intracellular regulation. *Proceedings of the National Academy of Sciences*, 97:7148–7153, 2000.

Richard Pearson, Young Kyeung Kim, Joseph Hokello, Kara Lassen, Julia Friedman, Mudit Tyagi, and Jonathan Karn. Epigenetic silencing of human immunodeficiency virus (HIV) transcription by formation of restrictive chromatin structures at the viral long terminal repeat drives the progressive entry of HIV into latency. *Journal of Virology*, 82:12291–12303, 2008.

Alan S. Perelson, Avidan U. Neumann, Martin Markowitz, John M. Leonard, and David D. Ho. HIV-1 dynamics in vivo: Virion clearance rate, infected cell life-span, and viral generation time. *Science*, 271:1582–1586, 1996.

Douglas D. Richman, David M. Margolis, Martin Delaney, Warner C. Greene, Daria Hazuda, and Roger J. Pomerantz. The challenge of finding a cure for HIV infection. *Science*, 323:1304–1307, 2009.

Kevin R. Sanft, Sheng Wu, Min Roh, Jin Fu, Rone Kwei Lim, and Linda Petzold. Stochkit2: software for discrete stochastic simulation of biochemical systems with events. *Bioinformatics*, 27:2457–2458, 2011.

Nilufer Seth, Daniel Kaufmann, Timothy Lahey, Eric S. Rosenberg, and Kai W. Wucherpfennig. Expansion and contraction of HIV-specific CD4 T cells with short bursts of viremia, but physical loss of the majority of these cells with sustained viral replication. *Journal of Immunology*, 175:6948–6958, 2005.

V. Shahrezaei and P. S. Swain. Analytical distributions for stochastic gene expression. *Proceedings of the National Academy of Sciences*, 105:17256–17261, 2008.

Velia Siciliano, Immacolata Garzill, Chiara Fracassi, Stefania Criscuolo, Simona Ventre, and Diego di Bernardo. miRNAs confer phenotypic robustness to gene networks by suppressing biological noise. *Nature Communication*, 4:2364, 2013.

Abhyudai Singh. Negative feedback through mRNA provides the best control of gene-expression noise. *IEEE Transactions on NanoBioscience*, 10:194–200, 2011.

Abhyudai Singh. Stochastic analysis of genetic feedback circuit controlling HIV cell-fate decision. *2012 IEEE Conference on Decision and Control*, pages 4918–4923, 2012.

Abhyudai Singh and João Pedro Hespanha. Optimal feedback strength for noise suppression in autoregulatory gene networks. *Biophysical Journal*, 96:4013–4023, 2009.

Abhyudai Singh and Mohammad Soltani. Quantifying intrinsic and extrinsic variability in stochastic gene expression models. *PLoS ONE*, 8:e84301, 2013.

Abhyudai Singh and Leor S. Weinberger. Stochastic gene expression as a molecular switch for viral latency. *Current Opinion in Microbiology*, 12:460–466, 2009.

Miguel Stevens, Erik De Clercq, and Jan Balzarini. The regulation of HIV-1 transcription: Molecular targets for chemotherapeutic intervention. *Medicinal Research Reviews*, 26:595–625, 2006.

Robinson Triboulet, Bernard Mari, Yea-Lih Lin, Christine Chable-Bessia, Yamina Bennasser, Kevin Lebrigand, Bruno Cardinaud, Thomas Maurin, Pascal Barbry, Vincent Baillat, Jacques Reynes, Pierre Corbeau, Kuan-Teh Jeang, and Monsef Benkirane. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science*, 315:1579–1582, 2007.

Zhiwei Wanga, Zhonghuai Houb, Houwen Xinb, and Zhizhou Zhanga. Engineered internal noise stochastic resonator in gene network: A model study. *Biophysical Chemistry*, 125:281–285, 2007.

Leor S Weinberger and Thomas Shenk. An HIV feedback resistor: Auto-regulatory circuit deactivator and noise buffer. *PLoS Biol*, 5:e9, 2006.

Leor S Weinberger, J Burnett, J Toettcher, A Arkin, and D Schaffer. Stochastic gene expression in a lentiviral positive-feedback loop: HIV-1 tat fluctuations drive phenotypic diversity. *Cell*, 122:169–182, 2005.

Leor S Weinberger, R.D. Dar, and M.L. Simpson. Transient-mediated fate determination in a transcriptional circuit of HIV. *Nature Genetics*, 40:466–470, 2008.