345f Stochastic Gene Expression in a Lentiviral Positive Feedback Loop: HIV-1 Tat Fluctuations Drive Phenotypic Diversity

Leor S. Weinberger, John C. Burnett, Jared E. Toettcher, Adam P. Arkin, and David V. Schaffer

HIV, the retrovirus that causes AIDS, has escalated into a global pandemic that has infected more than 38 million people and taken the lives of over 25 million. Although small molecule antiretroviral therapy for HIV has developed immensely over the past 20 years, the current treatments are unable to eradicate the virus from the host due to latent reservoirs of virus infected in a small fraction of quiescent immune (T) cells. In particular, post-integration latent reservoirs are composed of viral DNA integrated into the genome of a long-lived memory T cell population that for uncharacterized reasons remains inactive. This reservoir poses a major barrier to HIV treatment because the host cells are not actively transcribing the HIV genome but may reactivate to reseed viremia at any time, meaning that patients must continue antiretroviral therapies throughout their lives. The mechanism for establishing latent reservoirs remains unknown; however, the time immediately after infection appears to be critical in either the active replication of HIV (which leads to rapid cell death) or the silencing of HIV transcription (which may lead to viral latency).

We are conducting a rigorous experimental and computational analysis on the dynamics of gene expression in a model HIV lentivirus. In particularly, efficient HIV transcriptional regulation requires the viral protein Tat, which is itself encoded in the HIV genome and therefore represents a strong positive feedback loop/circuit. Genetic circuits are ubiquitous in living organisms, and positive feedback circuits can often attain two distinct states (active and inactive). Moreover, in genetic systems involving slow reactions and small numbers of molecules (such as a single copy of an integrated virus), sources of noise may exert important influences on the behavior of the circuit to contribute in switching between an active or inactive state.

We hypothesize that the establishment of the small reservoirs of HIV infected cells begins with a switch-like decision that occurs immediately after integration. Furthermore, we hypothesize that this switch is dependent on the concentration of Tat in the cell, and at low concentrations of Tat shortly after viral infection this switch becomes stochastic. We have developed a simple computational stochastic model of the Tat-mediated positive feedback circuit to examine our hypothesis. Moreover, we have designed an experimental model that incorporates that Tat positive feedback circuit of HIV with a green fluorescent protein (GFP) marker that allows us to indirectly monitor the concentrations of Tat and the transcriptional activity of HIV in the system. This combined experimental and computational system provides strong evidence that mammalian gene expression is stochastic and that stochastic effects in HIV gene regulation can contribute to the establishment of latent viral reservoirs. Finally, this fundamental work provides groundwork for the development of novel anti-HIV therapies.