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Single-Molecule Threshold of HIV Fate Decision

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ABSTRACT During early infection, the HIV virus makes a key decision between two states: lytic and lysogenic fate. Deterministic bistability requires combination of positive feedback and ultrasensitivity. Although HIV circuity includes positive feedback activation of the Tat transactivator, it lacks ultrasensitivity. How does the HIV circuit allow for multiple fates without ultrasensitivity? A new article suggests that HIV bistability is a result of a transient threshold that allows the kinetic trapping of the inactive state. Interestingly, the model shows that the transient threshold is a result of a single molecule threshold that occurs when the promoter toggles between inactive and active states.

The relationship between structure and function is fundamental to our understanding of biological systems. The structure-function relationship is critical at all scales, from organ physiology to cellular function and down to macromolecules. The structure-function paradigm is at the core of our understanding of biological systems. Evolution often reuses similar structures to solve the same problem and implement the same function. Biological networks and regulatory circuits follow the same paradigm; their structure often indicates their function. Specific circuit motifs recur over and over because, in many cases, biological systems face the same tasks and perform very similar functions. Evidence for recurring biological motifs emerged from the top-down analysis of motif enrichment in complex networks (1) and from the bottom-up analysis of what motifs can be used to address specific functionalities (2).

The ability to make a binary fate decision that allows for a qualitative change in output with only a small change in input is a ubiquitous task needed by many biological systems. In such systems, small fluctuations of input signals are suppressed until a specific threshold is crossed, after which the system transitions to a qualitatively new state. This type of functionality is at the core of many cellular decisions including proliferation, differentiation, or even death (3). Binary fate decisions are often implemented by a specific circuit structure: a combination of a positive feedback loop with a nonlinear ultrasensitive dose response (4). In such circuits there are two stable states and the transition between states occurs when the input crosses an activation threshold. To allow for bistability, both positive feedback and ultrasensitive activation are required components in the structure of this circuit. Without ultrasensitivity, the system will only have a single stable state at high activity levels. The well-established structure-function relationship among bistability, positive feedback, and ultrasensitivity often results in the implicit assumption that, if there is functional bistability and a positive feedback, the system will exhibit an ultrasensitive activation.

During early infection, the HIV virus makes a key decision between lytic and lysogenic fates. That decision has remarkable biomedical implications. The HIV epidemic has been largely suppressed through the development of highly active antiretroviral therapy (HAART). HAART treatment allows patients to suppress the disease to levels that are below the standard detection limit. Yet, whereas these patients show no symptoms or detectable viral load, they carry latent viruses at <1 in 10^6 CD4+ T-cells (5). A lapse in HAART treatment can cause resurgence of the disease and, therefore, the costly therapy must continue for the lifetime of the patient, a challenge in developing countries.

Given that the HIV virus makes a critical binary cell fate decision, many researchers naturally hypothesized that the network structure that implements this function similarly combines a positive feedback loop with ultrasensitive activation. One of these two elements has already been established: the Tat transactivator acts in a positive feedback to induce its own expression. Tat is a unique transcriptional enhancer that binds to an RNA stem-loop structure within a secondary structure of newly transcribed viral RNA, which encodes Tat itself,

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and enhances the transcription rate of viral RNA (6). The combination of a positive feedback motif structure with the two-state fate-decision function caused many researchers to speculate that a bistable system that is based on positive feedback and an ultrasensitive response implements the HIV-fate decision. However, a new article by Aull et al. (7) shows that this is not the case.

Aull et al. (7) take advantage of synthetic circuits where the underlying Tat feedback strength can be manipulated through small molecules that control the Tat transactivator synthesis and degradation rates. By manipulating feedback strength they directly tested the existence of hysteresis, a hallmark of deterministic bistable systems. Interestingly, they found no hysteresis in the system, supporting the notion that, although there is a clear activation threshold, it is not a result of deterministic bistability.

What is the exact mechanism that makes the HIV-fate decision? To address this question, they analyzed multiple models of HIV activation with increasing complexity. They show that a stochastic model of Tat activation that includes promoter toggling recapitulates the transient threshold phenomena. Furthermore, the timing of that threshold is a result of the number of activation steps required. It is interesting to note that the models only work when the integer number of molecules is considered, effectively arguing that a single molecule is the threshold. Overall, the model demonstrates how transient threshold can arise from a simple model of stochastic activation. Because epigenetic silencing of the latent state can act to stabilize latency, a stochastic slow rate of the initial Tat positive feedback loop can create a kinetic trap to allow the latent state enough time to epigenetically silence viral RNA expression.

Viruses can be thought of as the ultimate "hackers" of their host, where the host machinery is "pwned" to the needs of the virus. Although often detrimental to the host, our basic understanding of gene expression is indebted to understanding how viruses manipulate gene expression machinery. And although this work is focused on one such system, it is potentially applicable to any inducible promoter. Therefore, once again, analysis of how viruses "hack" an expression system allows us to answer questions about the fundamental mechanism of gene expression.

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