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Stochastic Fate Selection in HIV-Infected Patients

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Classic studies proposed that stochastic variability (“noise”) can drive biological fate switching, enhancing evolutionary success. Now, Ho et al. report that HIV’s reactivation from dormant (latently infected) patient cells—the major barrier to an HIV cure—is inherently stochastic. Eradicating an incompletely inducible (probabilistic) viral phenotype will require inventive approaches.

From tiny viruses to complex vertebrates, biological systems share a common challenge to preserve reproductive fitness in unpredictable, changing environments. Faced with environmental variability, many organisms evolve complex sensor-actuators to continually gauge their surroundings and deterministically adapt. But, 50 years ago, Dan Cohen proposed an alternate solution: if organisms could stochastically generate a range of phenotypes in each environment, they could “hedge their bets” in much the same way that financial houses diversify their assets to minimize risk against economic crashes. In desert annuals, where reproductive success is governed by unpredictable weather patterns, Cohen noted that fitness could be enhanced if chance governed each seed’s fate to germinate or hibernate (e.g., when the husk thickness of each seed is allowed to stochastically vary). With some seeds randomly entering dormancy whatever the environment, the annuals are always left with a long-lived subpopulation to avoid extinction during unforeseen droughts. But what molecular mechanism would allow organisms to probabilistically generate the needed cell-to-cell variability? Years later, studies of active-versus-dormant infection (i.e., lysis-lysogeny) in the bacterial virus phage λ suggested an answer: noisy gene expression (Arkin et al., 1998). Gene expression is, in fact, inherently stochastic, subject to random fluctuations in regulating enzymes, mRNAs, and other biomolecules. These diffusion-driven molecular fluctuations are unavoidable at the single-cell level and appear sufficient to shift cells between transcriptional on and off states (Raj and van Oudenaarden, 2008). With some cells randomly active and others dormant, the result is a distribution of cell fates across a population. A similar distribution of cell fates may now have been found in HIV patients in the clinic. In this issue of *Cell*, Ho and colleagues (Ho et al., 2013) report that HIV’s reactivation from lifelong dormant (i.e., “latent”) reservoirs is stochastic, likely interfering with persistent therapeutic efforts to activate and purge this problematic reservoir.

The theory that stochastic noise is sufficient to drive cell-fate decisions has been demonstrated in a range of biological systems, from bacteria to vertebrates (Balázsi et al., 2011). Nevertheless, the theory has faced stiff challenge. Alternative hypotheses have argued that hidden deterministic variables, for example the state of the host cell during viral infection or the number of infecting viruses, have a larger impact on eventual cell-fate (St-Pierre and Endy, 2008; Zeng et al., 2010). Unknown and unmeasured, these variables might strongly differ between the disparate cellular phenotypes, in fact predicting the seeming stochasticity. Ever finer and more expansive measurements, it appeared, would be needed to rule out deterministic explanations.

Unexpectedly, a new chapter may now come from a clinical angle. Much like bacteriophage λ, when HIV infects a cell, two outcomes are possible. After HIV integrates its proviral DNA into the genome of $CD4^+$ T cells, either it enters a state of active replication killing the cell or it enters a long-lived latent state where

transcription from the provirus is largely quiescent (Finzi et al., 1997). Since these latent cells are unaffected by antiretroviral therapy, which only targets actively replicating HIV, the latent reservoir ensures HIV's lifelong persistence during therapy. Most troublingly, latency is reversible, especially when latently infected cells subsequently activate. As a result, if a patient is removed from therapy, HIV levels quickly resurge from the progeny of reactivated latent viruses, rapidly reaching pretreatment levels (Figure 1A).

With latency being the greatest barrier to curing HIV in patients, the field has continually searched for a set of cellular drivers that might deterministically push HIV into or out of latency. The dominant view has been that latency results from HIV's infrequent infection of "transitioning" T cells, i.e., T cells undergoing the transition from activated to resting-memory states (Figure 1B, left). Insufficiently activated, these cells would not support viral gene expression, due to blocks such as heterochromatin-mediated silencing or a lack of transcriptional activators, for an excellent review see Siliciano and Greene (2011). Yet, evidence had also been found implicating HIV's own noisy transcriptional positive-feedback circuit in the latency decision (Weinberger et al., 2005). Stochastic depletions in a critical HIV molecule (Tat protein) can prevent active HIV transcription, resulting in a chance of latent infections whatever the target-cell state (Figure 1B, right).

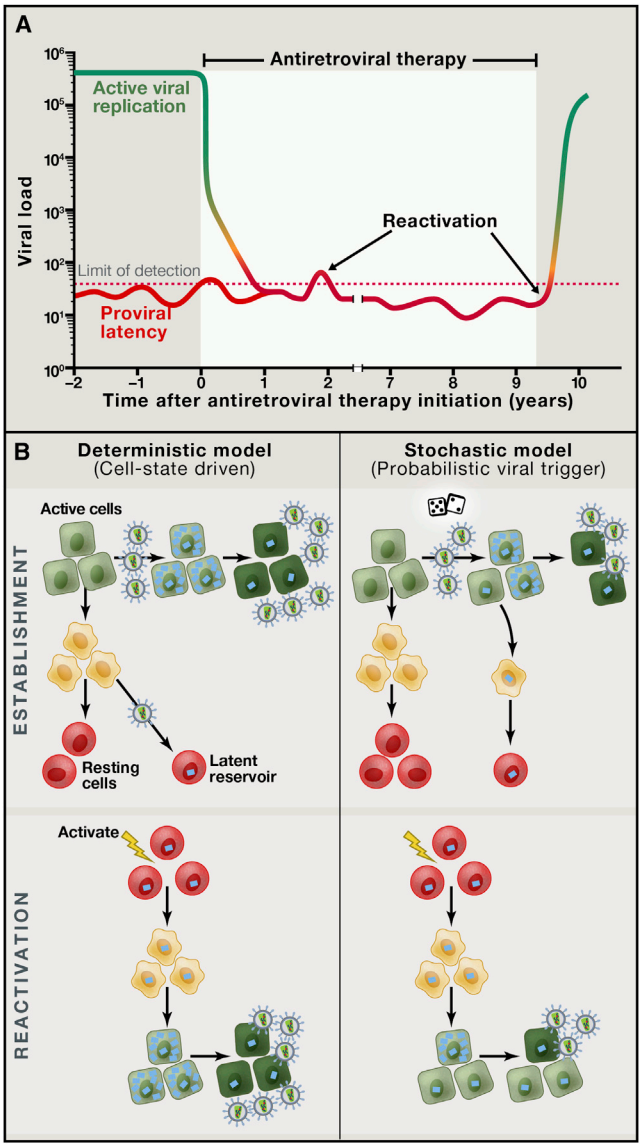

Figure 1. HIV Latency: Causes and Consequences

(A) A long-lived population of latently infected T cells persists below standard detection limits in patients and is not targeted by antiretroviral therapy. When therapy is halted, reactivating latent infections restore viral loads to pretreatment levels within weeks.

(B) Two generalized models for the establishment of latency and reactivation. Left: the previously prevailing deterministic model, in which the cellular-activation state determines whether HIV enters or exits latency. The deterministic model predicts that induction of a latently infected cell from a resting to an activated state results in reactivation of all latent virus. Right: an alternative model, proposing that latency is a stochastic viral decision. Each viral infection is a roll of the dice, with some probability of entering latency whatever the cellular activation state. In contrast to the deterministic model, the stochastic model predicts that viral reactivation from latency is probabilistic even when resting cells become fully activated.

Ho and colleagues did not set out to resolve the stochastic-versus-deterministic argument. As discoverers of HIV's latent reservoir over 15 years ago (Finzi et al., 1997), the Siliciano group sought to better calculate the latent reservoir's size. The standard assay for quantifying the reservoir isolates memory $CD4^+$ T cells from a patient and activates these cells to reactivate latent virus. By supplying the reactivating latent viruses with abundant target cells, infections can be scored and the number of latently infected cells back-calculated. However, not all integrated HIV is induced by cellular activation. Previously, these noninduced viruses were assumed to be defective, a result of HIV's high mutation rate. But, when Ho et al. sequenced these noninduced viruses, 12% of the genomes showed no obvious deletions or inactivating mutations. Moreover, when they synthesized the noninduced intact proviruses, the viruses grew with wild-type kinetics. They went further, analyzing many of the usual suspects of HIV latency, including: viral promoter function, promoter methylation, and viral integration into transcriptionally silent regions. None of these deterministic factors could explain why some viruses were noninduced while seemingly identical viruses were easily reactivated.

As a final test to determine if noninduced proviruses were in fact viable—since maximal cellular induction did not activate these viruses—Ho et al. performed beautiful repeated-stimulation experiments on the patient cells. By all measures, the first stimulation activated 100% of the cells from resting to activated. If latency were a deterministic epiphenomenon governed by cellular activation, or noninduced viruses were nonviable, subsequent stimulations on a completely activated target-cell population would have had little effect on the noninduced virus. Instead, during the second set of stimulation experiments, a subset of patient samples showed significantly more viruses reactivating from latency.

While it is always possible that "hidden" deterministic factors were missed, the fact that the same inputs generated distinct outputs is a hallmark of stochasticity.

By cementing stochasticity as a driver of HIV latency, this study may force a reevaluation of clinical attempts to purge the latent reservoir. First, it appears that the size of the latent reservoir has been underestimated, perhaps substantially. This is because previous studies assumed that, upon cellular activation, any noninduced viruses would be defective rather than latent. More critically, in presuming that cellular activation induces all latent viruses, many thought that the latent population could be deterministically purged. Touting an intervention known as "shock and kill," the idea was to first activate ("shock") patient cells to induce all latent virus. Standard antiretroviral therapy would then purge ("kill") the reactivated viruses, leaving a patient HIV-free. Unfortunately, it now appears that even the most potent "shocks" only reactivate a subset of the latent viruses. Perhaps repeated shocking will be more effective, but each repetition might just be another stochastic roll of the dice. Some virus will likely always emerge latent.

For basic virology, these findings raise the striking possibility that stochastic latency evolved to provide retroviruses like HIV with a bet-hedging fitness advantage. This would represent a paradigm shift in retrovirology where latency is currently viewed as a host-driven epiphenomenon with no evolutionary role in the natural history of infection. Viewing latency as an advantageous evolutionary fate decision—much as bacterial persistence and phage lysogeny are viewed—might explain why HIV Tat expression is exceptionally noisy, so noisy that Tat fluctuations alone are sufficient to drive a latency decision in nontransitioning cells (Weinberger et al., 2005). Given HIV's extremely rapid evolution, this noise would likely have been filtered out over the millions of years of natural lentiviral infections were it not selectively beneficial. But, how would noise be advantageous to lentiviruses? Stochastic latency would only provide a bet-hedging fitness advantage if lentiviruses needed to minimize their risks of extinction due to environmental catastrophes. In reality, lentiviruses mutate rapidly enough to evade immune clearance, generate extremely high viral loads, and only infect a small percentage (1%–2%) of environmental target cells. There appears to be little danger of lentiviral population crashes (and lentiviruses clearly did not evolve under pressure from antiretroviral drugs). If latency is, in fact, a viral-mediated stochastic fate decision, one wonders what selection pressures drive its persistence. Future work may address this.

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Stochastic Responses Are Not Left to Pure "Chance"

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Many coregulated genes assemble in multigene complexes via stochastic inter- and intrachromosomal interactions. In this issue, Fanucchi et al. report that chromatin loop formation governs hierarchical cotranscription within a multigene complex.

Cells are reliably informed about their environment via intracellular signaling pathways, which transfer chemical information in a chain reaction of events, involving conformational changes of receptors and other adaptor proteins, activation of enzymes, and finally, the assembly of transcriptional activating or repressing complexes. The unexpected