

JULY 29, 2005

Random Gene Expression May Drive HIV into Hiding

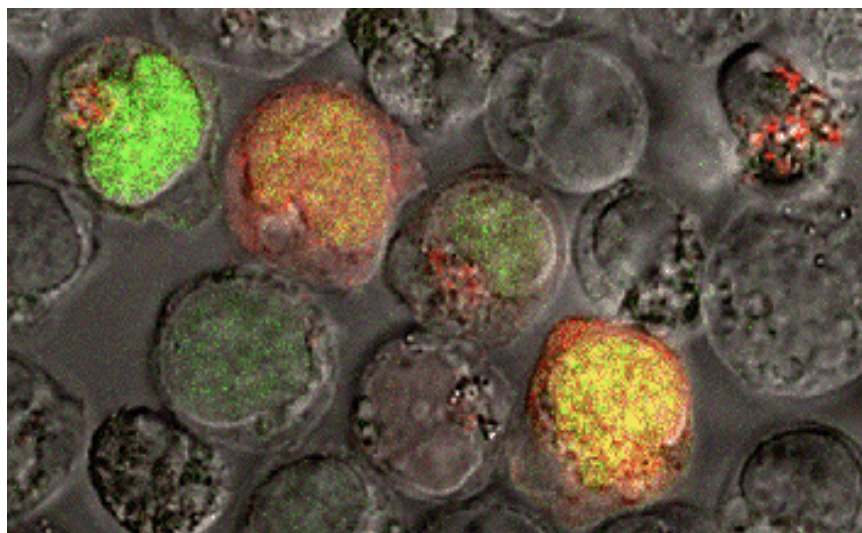


Image Title: A single cell was infected with an HIV Tat model virus containing green fluorescent protein (GFP) and grown into a population of clones. The virus is integrated into a single identical genomic position in all cells, but the cells display highly variable levels of GFP, which are driven by random fluctuations in HIV-1 Tat protein. - Leor Weinberger

Random fluctuations in gene expression can influence the fates of cells infected with human immunodeficiency virus (HIV) far more than previously thought, according to new research from Howard Hughes Medical Institute (HHMI) researchers at the University of California, Berkeley. By combining experimental and computational studies of HIV's replication cycle, the researchers found evidence that the virus may become latent in some cells by harnessing the random molecular behavior of the cell.

HIV can hide in cells for years before reappearing to make new virus. Latency is considered one of the biggest reasons why drug therapy fails to eradicate HIV from patients. The new findings, which will be published in the July 29, 2005, issue of the journal *Cell*, could help scientists design new and more effective treatments to slow or halt the progression of HIV infection.

HIV normally replicates rapidly in the body's white blood cells, but, in some cells, the virus stops replicating and becomes dormant. Researchers have long puzzled over how HIV makes the “decision” to become latent or to keep replicating in a certain cell.

"Any cell that gets infected can go down one of two paths."

- Leor Weinberger

“Most of the other groups studying the molecular mechanisms of HIV latency were coming at it from a deterministic point of view based on the belief that the system can only act in a predetermined way,” said lead author Leor Weinberger, who conducted the research as an HHMI predoctoral fellow in the laboratory of HHMI investigator Adam Arkin, in close collaboration with David Schaffer in the University of California, Berkeley, Department of Chemical Engineering. But the new results show that “any cell that gets infected can go down one of two paths,” Weinberger said.

Weinberger now is a postdoctoral fellow at Princeton University.

Inspired by work that Arkin had done in the 1990s, showing that random fluctuations in viral gene expression can influence latency in a bacterial virus, Weinberger wanted to see if the same process could be at work in HIV. No one had ever shown that this type of “noise” in gene expression could influence phenotype in infected mammalian cells, Weinberger said.

“I thought it was a cool idea,” he added, but “at the time, there wasn't a lot of data to support it. It was pretty far out there.”

Weinberger, Arkin, and their colleagues created a model HIV-1 vector—a virus that could enter human cells, carrying with it a key component of HIV's replication machinery: a gene called Tat. Tat facilitates transcription of HIV's entire genome, including itself, which creates a positive-feedback loop: If a little bit of Tat is around, then the HIV genome is transcribed efficiently, which makes more Tat, and so on. If a cell has no Tat, then the HIV genome may remain untranscribed and unable to replicate, so the virus heads for latency.

When the scientists infected cultured human cells with their viral vector, they found that cells that initially expressed a low level of virus were very unstable: After a few days, all cells expressed either a high level of virus or none at all.

When Weinberger took one infected human cell with low levels of the virus and allowed it to proliferate into many genetically identical copies of itself, he found that these progeny did not all show the same behavior: some turned their viral expression on high and others turned it off.

This dichotomy in cell fate from genetically identical cells is consistent with the idea that random fluctuations in gene expression control what happens to the cells, the researchers said. To see if this really was the best explanation, they conducted a large array of experimental controls to discount other hypotheses and then created computer programs to model what would happen to the virus under different cellular conditions.

Of the 16 models they tested, only one produced results that matched those seen experimentally in the infected cells. In this model, after HIV's RNA enters a host cell, it is copied into DNA, and then a little bit of Tat is made before the virus integrates into the host genome.

At this point, Tat must be chemically modified before it can encourage transcription of more HIV, and random thermal fluctuations in the cell can influence if and when these chemical modifications take place.

Because of Tat's positive-feedback loop, "these fluctuations can be amplified and can lead to very different qualitative behaviors," said Arkin.

If the appropriate modifications take place, then the HIV genome is transcribed and the positive feedback loop kicks in. If these Tat modifications don't happen, then HIV ceases to be expressed, and the cell can then possibly enter a latent state.

The significance of fluctuations in expression are dependent on HIV being expressed at a low level in the cell initially, Arkin said. Commonly, it's only when just a few molecules are interacting with each other that random fluctuations can have such a large effect on eventual outcome, he explained.

The researchers hope that understanding the molecular basis of HIV latency will lead to new treatments to slow or stop progression to AIDS. For example, Arkin suggested, the analysis implies that it might be effective to target the chemical modifications that Tat must undergo before it allows more HIV to be made. "When you quantify things and dissect them at this level, it gives you ways of exploring where your most vulnerable places might be."

Commenting on the work in a preview article published in the same issue of *Cell*, William J. Blake and James J. Collins of the Center for Biodynamics of Boston University, wrote: "The work of Weinberger et. al. represents an important step in moving from studies that elucidate the origins of stochasticity in gene expression to those that investigate the consequences of such molecular noise on cellular function. The authors [present] a scenario in which HIV-1 can hedge its bets by having an inherent ability to proceed

either to latency or viral production. This intriguing notion still needs to be tested experimentally, and more broadly, much work remains to be done to understand the functional role that gene expression noise potentially plays in the progression of disease.”