

Deep-sleeping HIV genomes under control

Nicolas Chomont

In a few people living with HIV, the virus remains under control without antiretroviral therapy. It emerges that, in these people, the viral DNA that is integrated into the host genome is in a deeply transcriptionally repressed state. See p.261

Our ability to keep HIV under control has been revolutionized by antiretroviral therapy (ART). But ART is not a cure – the HIV genome can integrate into host DNA and hide out in cells in a silent form, even after decades of successful therapy^{1–3}. ART must be continued throughout life, to prevent the virus from rebounding from these viral reservoirs. Could ways to prevent this viral rebound be found by studying the small proportion (less than 0.5%) of people living with HIV who can control viral replication without the need for ART? On page 261, Jiang *et al.*⁴ compared the viral reservoirs of these individuals, known as elite controllers, with those of people who are prescribed ART. Their findings suggest that elite control is associated with a small reservoir from which HIV is unlikely to be reactivated.

The authors began by using a sophisticated sequencing technique to compare viral genomes (proviruses) in millions of cells from the two groups of people. As expected, the comparison revealed fewer copies of the HIV genome in elite controllers than in people receiving ART. However, a higher proportion of the proviruses found in controllers were genetically intact – meaning that they have the potential to generate infectious viral particles when transcribed.

Jiang *et al.* frequently observed many identical copies of the viral genome in elite controllers. This observation confirms⁵ that infected cells have the ability to proliferate in controllers, as they do in people receiving ART^{6–8}. Elite controllers are known⁹ to mount a potent immune response against HIV-infected cells, and the authors found that the proviral sequences persisting in elite controllers were predicted to generate viral proteins that could be targeted by this response.

How, then, do these proviruses escape the immune response? To answer this question, the authors made use of a recently developed approach¹⁰ to analyse the sites at which viruses have integrated into the host genome, in conjunction with corresponding proviral sequences. The analysis revealed

several characteristics that suggest that the proviruses found in elite controllers are in a deeper state of latency (dormancy) than are the proviruses in people treated with ART.

First, proviruses in elite controllers are more likely to be integrated in non-protein-coding regions of the genome. Second, viral genomes from controllers are frequently positioned in, or surrounded by, repetitive stretches of DNA at chromosomal structures called centromeres. The host genome is packaged into a DNA–protein complex called chromatin – at centromeres, this packaging is unusually dense, which strongly represses transcription. Third, a substantial portion of HIV genomes in elite controllers are integrated in genes that encode members of the zinc-finger protein family, at which chromatin notoriously carries many molecular modifications that are associated with transcriptional repression¹¹.

The authors also performed an analysis of accessible chromatin regions (those at which

transcription is possible), which revealed that virus-integration sites in the DNA of elite controllers are located significantly farther from accessible chromatin than those in ART-treated individuals. This result reinforces the idea that the genomes of elite controllers are less likely to actively produce viral transcripts and proteins. Indeed, intact proviruses in elite controllers produced ten times fewer viral transcripts than did HIV genomes from people receiving ART.

Two scenarios could explain the peculiar proviral landscape of elite controllers. First, HIV integration could preferentially occur in particular regions of the genomes in these individuals. Alternatively, the proviruses that integrate into non-coding or transcriptionally repressed regions could be selected over time, with those that are more permissive to viral transcription being eliminated.

Definitively distinguishing between these two possibilities would require researchers to follow elite controllers over a long period of time, which was not within the scope of the current study. However, when Jiang *et al.* infected cells from elite controllers and people receiving ART with HIV *in vitro*, they found no significant difference in the integration patterns between the two, making the first scenario unlikely. The second model is also attractive because of the unusually potent immune responses against HIV-infected cells frequently observed in elite controllers. These responses might gradually eliminate the provirus-containing cells that are more likely to produce viral proteins (Fig. 1). Such selection could, over years, result in a reservoir made entirely of proviruses that are unlikely to be reactivated.

This idea is supported by previous work¹² indicating that the pool of replication-

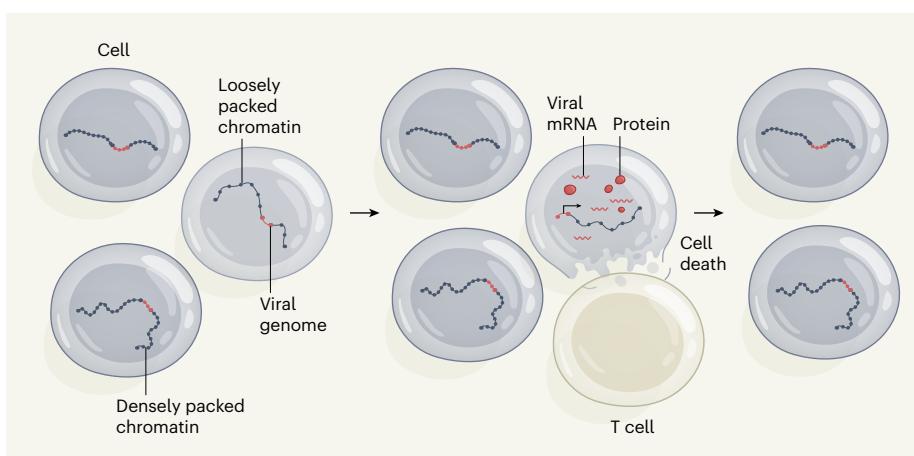


Figure 1 | Selection of sleeping HIV in elite controllers. A small proportion of people living with HIV can control the virus without antiretroviral therapy (ART). Jiang *et al.*⁴ provide evidence that the viral DNA in these elite controllers is integrated across the host genome. Some viral genomes become integrated at places in which host DNA is loosely packed with proteins in a complex called chromatin, meaning that transcription can occur. Other viral DNA is integrated at host sites where transcription is repressed because chromatin packaging is dense. Cells that transcribe the virus (generating viral messenger RNA and proteins) are efficiently targeted by immune T cells – a response seen only in elite controllers. These cells are killed, and so a small pool of cells harbouring deeply latent HIV genomes is evolutionarily selected over time.

competent virus is extremely small in elite controllers. Furthermore, one participant in Jiang and colleagues' study had no detectable replication-competent HIV at all, even though the authors thoroughly analysed more than one billion cells from this person. Whether HIV has been completely eradicated from this individual's body will be hard to demonstrate, but their case is certainly reminiscent of previous reports of HIV cure^{13,14}.

Elite controllers represent only a small proportion of people living with HIV. Nonetheless, Jiang and colleagues' work has several implications for the rest of this population. It suggests that deeply latent proviruses could preferentially persist after years of viral suppression with ART, particularly in individuals who have maintained immune responses against HIV. Perhaps continuous immune pressure over years would select a small reservoir from which HIV replication would be less likely to reignite. But whether deep-sleeping viral genomes could be reactivated and contribute to viral rebound during ART interruption remains to be determined.

Either way, the results of this study imply that both the intactness and the activation potential of viral genomes should be assessed when measuring the magnitude of the persistent HIV reservoir that can cause viral rebound. Assays that are currently used to estimate the size of the viral reservoir generally measure either the number of intact HIV genomes or their ability to generate RNA or proteins *in vitro*. Jiang and colleagues' work suggests that combining both measures could be necessary, because many intact genomes might not be easily reactivated. A combination measure could provide researchers and clinicians with a better predictor of viral rebound following ART interruption.

The study indicates that a continuous and prolonged cellular-immune pressure might substantially reduce the size of the HIV reservoir over time, by selecting a small pool of cells containing hard-to-reactivate HIV genomes. This, in turn, suggests that immune-cell therapies – including therapies based on CAR T cells, which are currently being developed to control HIV reservoirs¹⁵ – might not only control viral rebound during ART interruption, but also shrink the viral reservoir to a pool of deeply latent proviruses. Whether this could result in a long-term remission of HIV infection remains, of course, to be determined.

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1. Finzi, D. et al. *Science* **278**, 1295–1300 (1997).

2. Wong, J. K. et al. *Science* **278**, 1291–1295 (1997).

3. Chun, T. W. et al. *Proc. Natl. Acad. Sci. USA* **94**, 13193–13197 (1997).
4. Jiang, C. et al. *Nature* **585**, 261–267 (2020).
5. Boritz, E. A. et al. *Cell* **166**, 1004–1015 (2016).
6. Wagner, T. A. et al. *Science* **345**, 570–573 (2014).
7. Maldarelli, F. et al. *Science* **345**, 179–183 (2014).
8. Simonetti, F. R. et al. *Proc. Natl. Acad. Sci. USA* **113**, 1883–1888 (2016).
9. Migueles, S. A. et al. *Immunity* **29**, 1009–1021 (2008).
10. Einkauf, K. B. J. *Clin. Invest.* **129**, 988–998 (2019).
11. Vogel, M. J. et al. *Genome Res.* **16**, 1493–1504 (2006).
12. Blankson, J. N. et al. *J. Virol.* **81**, 2508–2518 (2007).
13. Hutter, G. et al. *N. Engl. J. Med.* **360**, 692–698 (2009).
14. Gupta, R. K. et al. *Nature* **568**, 244–248 (2019).
15. Herzig, E. et al. *Cell* **179**, 880–894.e10 (2019).

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Cell biology

The plant response to heat requires phase separation

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Temperature determines the geographical distribution of plants and their rate of growth and development, but how they sense high temperatures to mount a response was unclear. Now a process underlying this responsiveness is known. **See p.256**

Unlike animals, plants cannot move to escape harsh conditions. Consequently, they must continuously monitor their environment and, when exposed to high temperatures, quickly adjust their expression of developmental and growth-related genes. On page 256, Jung *et al.*¹ describe a molecular process that might underlie this temperature responsiveness.

The expression of developmental and growth-related genes in animals and plants typically occurs in a rhythmic fashion over a 24-hour cycle. Such daily oscillations are controlled by a molecular loop of protein activity that provides what is termed the circadian clock. Clock-induced transcriptional changes enable plants to anticipate daily environmental changes.

In the model plant species *Arabidopsis thaliana*, one component of the circadian clock is a protein assembly called the evening complex. It is maximally active at dusk and represses the expression of many genes important for plant development. The evening complex comprises the transcription-factor protein ELF3 (Fig. 1), a small peptide known as ELF4 and a protein called LUX. Plants with mutations that disable the gene encoding ELF3 flower earlier than normal during development and grow long embryonic stems termed hypocotyls, suggesting that ELF3 has a key developmental role.

Temperature fluctuations are known to affect the circadian rhythm of plants. The growth of *A. thaliana* at 22°C is normally restricted to the period around dawn, because of the repressive action of the evening complex at other times of day². However, at 27°C, this growth repression is relieved², and plants show accelerated flowering and rapid hypocotyl elongation compared with growth at

22°C. Yet the mechanism underlying such temperature-regulated growth has remained a mystery. Jung and colleagues propose that a physical process called phase separation is at the heart of plant responsiveness to heat.

To investigate, the authors focused on ELF3. They engineered *A. thaliana* so that ELF3 was replaced with a related version from two plants that do not show temperature-accelerated flowering: *Solanum tuberosum* (potato) and *Brachypodium distachyon* (a grass). The resulting *A. thaliana* plants were indistinguishable from wild-type *A. thaliana* at moderate temperatures, but were unable to accelerate flowering at a higher temperature, suggesting that ELF3 has a key role in temperature responsiveness.

To investigate further, Jung and colleagues focused on a region of ELF3 that is enriched in polar (hydrophilic) amino-acid residues, depleted of charged residues and predicted to be intrinsically disordered. Such protein regions are known as prion-like domains (PrDs), and have been proposed to mediate environmental responses in budding yeast (*Saccharomyces cerevisiae*)^{3,4}. Jung *et al.* engineered *A. thaliana* to express a chimaeric protein, in which its normal PrD was replaced with the corresponding region of ELF3 from *B. distachyon*. The authors report that the engineered plant did not display temperature-accelerated flowering, indicating that this ELF3 domain might have a key role in establishing temperature responsiveness.

The PrD of *A. thaliana* contains continuous stretches of the amino acid glutamine that are called polyglutamine (polyQ) repeats. The authors note a correlation between plant species that have long polyQ repeats in this domain and accelerated growth at warm