Journal Club

TWO PRECIOUS LESSONS FROM THE HIV-1 RT STRUCTURE

I vividly remember when I decided to become a structural biologist. The epiphany occurred when, for a university assignment, I came across the revolutionary work of Kohlstaedt et al., published in *Science* in 1992. This paper reports the structure of the human immunodeficiency virus 1 reverse transcriptase (HIV-1 RT). For the first time, the enzyme performing the crucial 'reverse transcription' of the viral RNA genome into DNA was visualized at atomic resolution. HIV-1 RT is composed of two proteins, p61 and p55, arranged in a 'hand' shape, into which the RNA is accommodated to be replicated into DNA.

This work was a real game-changer, because it revealed the binding pocket of nevirapine, a drug that had shown promise in improving the survival of individuals with AIDS. Nevirapine throws sand in the gears of the reverse-ranscription machinery by binding to p61 outside of its RNA-binding surface, thereby preventing the enzyme from correctly 'grabbing' the RNA. Importantly, the binding site of nevirapine lies in a conserved region of HIV-1 RT, so the notoriously mutating virus is unlikely to develop resistance to it, a cause that is responsible for the loss of efficacy of other treatments.

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The structure of HIV-1 RT paved the way to the development of a class of drugs that, 28 years later, are still used to treat HIV. According to the World Health Organization, the work of Kohlstaedt et al. contributed to saving the lives of almost 26 million people (see who.int/gho/hiv/epidemic_response/ART/en/) as well as to reducing the stigma associated with contracting HIV and with AIDS, which I had witnessed growing up in the 1990s.

Kohlstaedt's work imparted two valuable lessons to me. It has taught me that a protein structure can have great medical potential, and that treatments can be found for the most vicious viruses, sometimes by looking in unanticipated places. These lessons represent an inspiration in the current, bizarre times of the COVID-19 pandemic.

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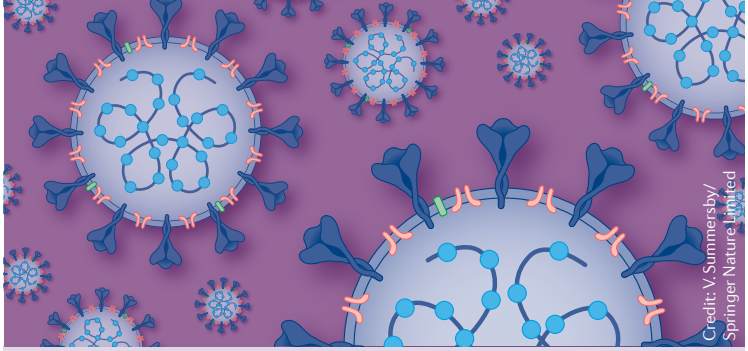
ORIGINAL ARTICLE Kohlstaedt, L. A. et al. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* **256**, 1783–1790 (1992)
RELATED ARTICLE Hu, W.-S. & Hughes, S. H. HIV-1 reverse transcription. *Cold Spring Harb. Perspect. Med.* **2**, a006882 (2012)



SARS-CoV-2

Cellular basis for SARS-CoV-2 infection

Research institutions, pharmaceutical companies and governmental rganizations are working to identify and develop drugs and vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19. The development of new therapies relies on the understanding of host–virus interactions and the biology of infection. In *Cell*, Daniloski, Jordan et al. report a genome-wide CRISPR–Cas9-mediated loss-of-function screen to identify host factors required for SARS-CoV-2 viral infection.

Their screen used human alveolar basal epithelial carcinoma cells ($A549^{ACE2}$) that ectopically express angiotensin converting-enzyme 2 (ACE2) — the host receptor to which viral envelope proteins bind and that is required for infection — and targeted 19,050 genes. As expected, the genes whose loss conferred resistance to SARS-CoV-2 included genes involved in viral entry and replication. Among the 50 most represented genes (top-ranking) were genes encoding proteins that function in complexes and/or in distinct pathways, prominently involved in endocytotic trafficking (for example the Retromer and Commander complexes).

To further validate their screen, the authors used other methods to perturb the function of top-ranking genes in $A549^{ACE2}$ cells: CRISPR knockout, siRNA knockdown, and small-molecule inhibitors that include some that are FDA approved or in phase II or phase III clinical trials for other diseases. Interestingly, some of the most effective inhibitors had an additive effect, increasing protection from SARS-CoV-2 infection when used in combination.

The authors also evaluated ACE2 cell surface expression following knockout of their top-ranking genes and found that ACE2 was substantially reduced in *RAB7A*-knockout cells. *RAB7A* encodes a small GTPase that regulates membrane trafficking and vesicular transport, and its knockout led to the accumulation of ACE2 in the cytoplasm and in vesicles similar to endo-lysosomes; however, the mechanisms by which RAB7A loss disrupts viral infection, which might involve multiple pathways, remain to be determined.

When looking at gene expression profiles (using single-cell transcriptomics) of cells in which the top-ranking genes were knocked out, the authors found that the CRISPR–Cas9-driven loss of six independent genes induced a similar transcriptional signature, which was associated with increased cholesterol synthesis. Indeed, cholesterol levels were higher in cells lacking each of these six genes. These findings are in agreement with another study reporting that cholesterol upregulation by pharmacological treatment might be a mechanism for viral inhibition.

Other studies are reporting loss-of-function screens to identify host factors required for SARS-CoV-2 infection — these studies, combined with protein–protein interaction network analyses and other large-scale screens, should provide useful resources for the development of therapeutic strategies against COVID-19.

Kim Baumann

ORIGINAL ARTICLE Daniloski, Z., Jordan, T. X. et al. Identification of required host factors for SARS-CoV-2 infection in human cells. *Cell* https://doi.org/10.1016/j.cell.2020.10.030 (2020)