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# Molecular espionage shows a single HIV enzyme's many tasks

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Using ingenious molecular espionage, scientists have found how a single key enzyme, seemingly the Swiss army knife in HIV's toolbox, differentiates and dynamically binds both DNA and RNA as part of the virus' fierce attack on host cells. The work is described this week in the journal *Nature*.

The enzyme, reverse transcriptase (RT), is already the target of two of the three major classes of existing anti-HIV drugs. The new work, using single-molecule fluorescent imaging to trace RT's activity in real time, not only reveals novel insights into how this critical viral enzyme functions, but also clarifies how some of the anti-HIV pharmaceuticals work.

The research team, at Harvard University and the National Cancer Institute, was led by Xiaowei Zhuang at Harvard and Stuart Le Grice at NCI. Elio A. Abbondanzieri at Harvard and Gregory Bokinsky, formerly at Harvard and now at the Lawrence Berkeley National Laboratory, are lead authors.

"Our experiments allowed us, for the first time, a peek at how individual RT molecules interact with the HIV genome," says Zhuang, professor of chemistry and chemical biology and of physics in Harvard's Faculty of Arts and Sciences, as well as an investigator with the Howard Hughes Medical Institute. "We found that RT binds RNA and DNA primers with opposite orientations and that RT's function is dictated by this binding orientation."

HIV begins its assault by injecting its single-stranded RNA into a host cell. Three subsequent steps are all mediated by RT: The viral RNA is converted into single-stranded DNA, the single-stranded DNA is replicated into double-stranded DNA, and the original viral RNA is degraded. Another enzyme mediates the final step of the genome conversion, where the viral double-stranded DNA is inserted into the host's DNA, allowing it to take advantage of the host's genetic machinery to replicate and propagate itself.

Using their molecular probe to spy on this process, Abbondanzieri and colleagues traced RT's multitasking skill to its dynamic active sites, which allow it to bind and process RNA as well as single- or double-stranded DNA.

"Remarkably, RT can spontaneously flip between these two opposite orientations on DNA and RNA to facilitate two distinct catalytic activities," says Abbondanzieri, a postdoctoral researcher in Harvard's Department of Chemistry and Chemical Biology. "These flipping motions, which have never before been seen in a protein-nucleic acid complex, can be likened to a nanoscale version of a gymnastics routine on a pommel horse."

The 180-degree flipping of RT is regulated by nonnucleoside RT inhibitors (NNRTIs), a major class of anti-HIV drugs. Abbondanzieri and coworkers observed NNRTIs inhibiting HIV activity by accelerating RT's flipping between its two active sites, hindering the enzyme's ability to convert single-stranded DNA to double-stranded DNA.

Source: Harvard University