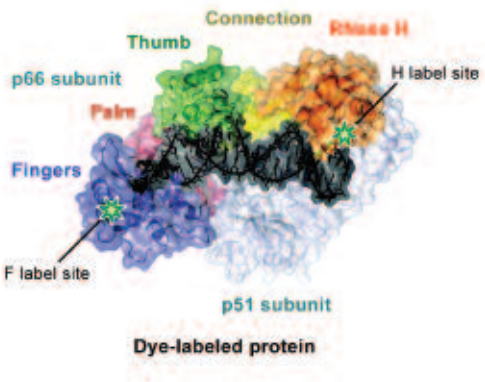
Reverse Transcriptase: When Function Follows Direction



(*Image: S. Le Grice, CCR*)

Getting into a host cell's genome can be a tough job for HIV. Before it can be integrated, the virus' single-stranded, RNA-based genome must undergo a complete transformation into double-stranded DNA, a multistep process that holds much room for error. This transformation is where HIV's multifunctional reverse transcriptase (RT) comes into play. RT does three things: transcribes the viral RNA (vRNA) into a single, complementary DNA (cDNA) strand; destroys the original vRNA; and uses the new cDNA as a template for a complete double-stranded viral DNA (vDNA). These three activities require that RT behave in two seemingly contradictory ways: as a polymerase and as an RNase H.

The direction in which HIV's reverse transcriptase binds to the viral genome determines whether the enzyme reads the RNA or degrades it. This knowledge has shed new light on the mechanisms by which antiviral drugs called non-nucleoside RT inhibitors act against HIV.

While the structural sites of RT's polymerase and RNase H activities are well known, the parameters that determine when and how each site is engaged are not. How does RT decide whether to enter polymerase or RNase H mode?

Using combinations of DNA- or RNA-based primers and templates, the groups of Harvard University's Xiaowei Zhuang, Ph.D., and Stuart Le Grice, Ph.D., Head of CCR's RT Biochemistry Section, have found that RT's function is controlled by its orientation.

In this system, when presented with a DNA primer bound to an RNA or DNA template—a scenario analogous to the production of a cDNA or vDNA strand—RT positions itself in a way that favors its polymerase site. However, when RT finds a short RNA primer bound to a longer stretch of DNA—which to the enzyme looks like a newly transcribed cDNA strand bound to its original vRNA template—it binds in the opposite orientation, promoting its RNA-template-degrading RNase H activity.

Remarkably, the researchers also found that when RT sees a substrate that is amenable to both of its functions, such as polypurine tracts (vRNA sequences that in nature serve as primers for reverse transcription of the HIV genome), the protein flips spontaneously between orientations, potentially helping it maximize the efficiency with which it transcribes and degrades the viral RNA. Exposure to non-nucleoside RT inhibitors (NNRTIs)—a class of potent and clinically approved anti-HIV drugs that bind to RT's polymerase site—significantly altered RT's flipping behavior, forcing the enzyme to adopt its RNase H-promoting orientation.

These data, published in the May 8, 2008, issue of the journal *Nature*, provide significant insights into the structural biology underlying the reverse transcription process in HIV and lend new evidence to the observation that NNRTIs can, in certain circumstances, prevent the synthesis of DNA by reversing enzyme orientation.