

OBESITY

What's your fat-cell allowance?

In the American sitcom *Friends*, Monica bakes mouth-watering cookies for her housemates, but — having been overweight as a teenager (pictured) — she is reluctant to eat them herself. Kirsty Spalding and colleagues now provide scientific evidence for why Monica has every reason to be cautious (K. L. Spalding *et al. Nature* doi:10.1038/nature06902; 2008).

Two factors contribute to an increase in fat mass: the number of fat cells and how much fat each of them stores (their volume). The authors studied the dynamics of fat-cell number in some 700 adults, both lean and obese, and combined their data with previous observations in children and adolescents.

A clear pattern emerged: irrespective of weight, the number of fat cells seems to rise steadily from birth to the early twenties, but remains constant thereafter. Moreover, in patients observed

before and up to two years after surgical treatments that facilitate weight loss by reducing stomach size, no decrease in fat-cell numbers was detected — although their volume did drop.

So, are fat cells that are generated in early life doomed to remain with us till death us do part? In animal studies, this question can be addressed by labelling DNA nucleotides with radioactive isotopes such as ^{14}C . Differentiated fat cells do not divide, and so radioisotopes, incorporated in their DNA in the last round of division before differentiation, remain there throughout the cells' life. The time of radiolabel incorporation, which is worked out from its half-life, is therefore the 'birth date' of these cells. But the potential toxicity of radioisotopes means that such studies cannot be performed in humans.

Spalding *et al.* cleverly thought of the next-best option. Atmospheric

levels of ^{14}C have remained relatively constant for centuries, with the only major increase occurring between 1955 and 1963, when nuclear bombs were being tested above ground. A chain of reactions ensures that, at any given time, the radioisotope content of human DNA matches that of the atmosphere. The authors could thus follow fat-cell dynamics in individuals born around 1955–63.

As Spalding and colleagues' results show, fat cells have a high turnover: new cells are continually being born to replace their dead predecessors. The average age of a fat cell seems to be about 10 years in both lean and obese individuals, and the number of fat cells as a proportion of all cells remains constant in each weight group. But the total number of new fat cells was higher in obese subjects, suggesting that they are replenishing an existing larger pool.

So do the lean among us need to worry about our diet if we have fewer fat cells? Yes, we do: our fewer fat cells can still store large amounts of fat. Also, can obese people do anything about their weight? After all, they've already accumulated a



large pool of fat cells in childhood and adolescence? Again, the answer is yes. As Monica seems to have guessed, they can still reduce the volume, if not the number, of their fat cells. A further corollary of the paper is that researchers seeking drugs to cure obesity should consider targeting the mechanisms underlying fat-cell turnover. **Sadaf Shadan**

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MOLECULAR BIOLOGY

An HIV secret uncovered

Eddy Arnold and Stefan G. Sarafianos

With two catalytic activities and many substrates, how does HIV's reverse transcriptase enzyme know what to do to which substrate? Zooming in on the enzyme's molecular interactions provides tantalizing clues.

To replicate within their host cell, retroviruses such as HIV must make a double-stranded DNA copy of their single-stranded RNA genome. This elegant process, by which a viral enzyme — reverse transcriptase — synthesizes some 20,000 nucleotides, with the flow of genetic information moving in the opposite direction to normal (DNA to RNA), is nothing short of amazing. It also provides numerous opportunities for therapeutic intervention, as reverse transcriptase is the target of nearly half of the drugs approved for treating AIDS. But reverse transcription is not simple, for the enzyme comes into contact with diverse nucleic-acid substrates (DNA and/or RNA). On page 184 of this issue, Abbondanzieri *et al.*¹ describe how this enzyme discriminates between substrates at intermediate steps of reverse transcription.

Reverse transcriptase has two distinct enzymatic activities: it is a DNA polymerase capable

of copying either an RNA or a DNA template into a complementary DNA sequence; and it is an RNase H, capable of degrading the RNA strand of an RNA–DNA duplex once it has been used as a template for the first DNA strand (called the 'minus' strand; Fig. 1). But the enzyme overlooks some RNA segments, called polypurine tracts (PPTs), sparing them from degradation; these segments have an unusual sequence (and so structure), which prevents the enzyme's RNase H domain from cutting them².

Like other DNA polymerase enzymes, reverse transcriptases initiate DNA synthesis using short nucleic-acid segments called primers. The primer used by HIV reverse transcriptase to generate the minus-strand DNA is a host-cell transfer RNA sequence. For the formation of the second (plus) DNA strand, which is complementary to the minus strand, the enzyme uses PPT segments that it has

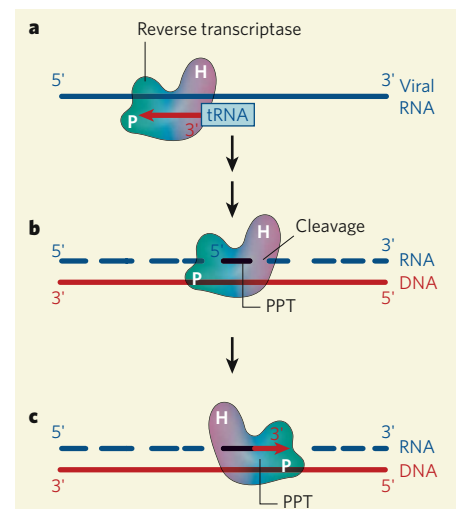


Figure 1 | Reverse transcription. Like other reverse transcriptase enzymes, that of HIV mediates transcription of the virus's RNA genome into double-stranded DNA. **a**, To form the first DNA strand (the minus strand) the enzyme uses a transfer RNA as a primer and interacts with the tRNA 3' end in a polymerase (P) binding mode. **b**, As the complementary minus-DNA sequence is being synthesized, the enzyme cleaves the RNA template (but leaves the PPT sequences of the RNA intact) by binding to it in an RNase H (H) mode. **c**, Finally, to initiate the synthesis of the second (plus) DNA strand, the reverse transcriptase uses the PPT sequence as a primer, once again binding in the polymerase mode.

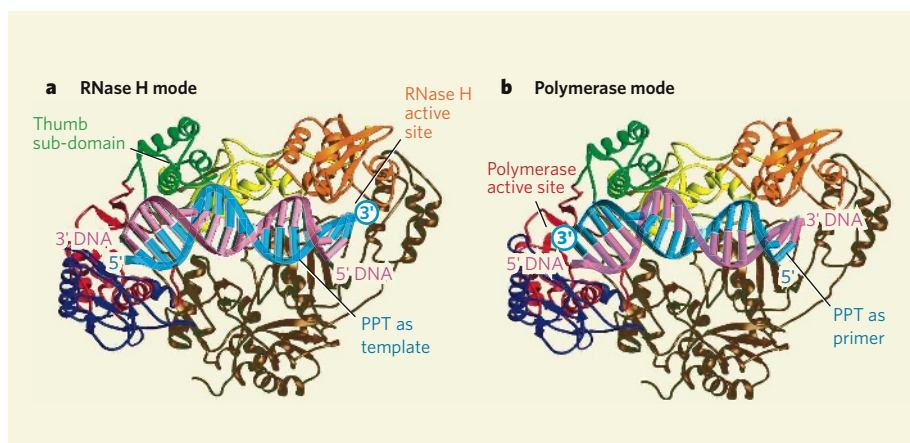


Figure 2 | The dual binding mode of HIV reverse transcriptase. As part of the virus's RNA genome, the PPT sequence acts as a template for the synthesis of the minus-strand DNA. **a**, Abbondanzieri *et al.*¹ show that, before cleavage of the PPT RNA, HIV reverse transcriptase binds to PPT (blue sequence) in an RNase H mode — that is, the RNase H domain of the enzyme is close to the 3' end of PPT (circled), thus blocking the polymerase domain's access to it and preventing premature extension. **b**, When the RNA nucleotides next to the PPT sequence are cleaved, allowing PPT to function as a primer, the enzyme can bind in a polymerase-competent mode (with its polymerase domain close to PPT's 3' end) to initiate DNA synthesis. The HIV reverse transcriptase seems to rapidly switch orientation between these two binding modes.

generated by cleaving the template RNA on both sides of these fragments (Fig. 1b).

The molecular details of the interactions between HIV reverse transcriptase and its various substrates have been revealed through structural analyses of the enzyme when in complex with nucleic acids (DNA–DNA or RNA–DNA strands)^{2,3}. They have also been studied by analyses of nucleic acids (DNA–DNA) in the presence of dNTPs (the incoming free nucleosides that join and extend a nucleic acid)⁴. Together with complementary biochemical studies, these structures showed that reverse transcriptase interacts with both nucleic acids and the incoming dNTPs when in the polymerization mode, with its polymerase active site aligned at one end (the 3' end) of a primer. When in the RNA-cleavage mode, it interacts with RNA–DNA, with the active site of the RNase H domain aligned with the RNA strand² (Fig. 1). A remaining puzzle has been how reverse transcriptase can distinguish PPT RNA, as an efficient primer for the initiation of second-strand-DNA synthesis, from the rest of the RNA sequence, which, having already been used as a template, is cleaved by RNase H.

Abbondanzieri *et al.*¹ present fascinating clues about the interactions between HIV reverse transcriptase and various nucleic-acid substrates. To determine the orientation in which individual enzyme molecules bind to an RNA–DNA hybrid, the authors used the technique of fluorescence resonance energy transfer (FRET). Typical biochemical experiments involve analysing populations of molecules or complexes. By contrast, single-molecule spectroscopy techniques such as FRET allow direct, time-dependent observation of individual molecular events. For FRET analysis, two molecules of interest — in this case, reverse transcriptase and the nucleic

acid — are labelled with fluorescent chemical groups, and the transfer of light energy from one molecule to the other, which depends on the distance between them, is used as a measure of their interactions.

The authors find that, when reverse transcriptase binds to a nucleic acid in which a relatively short RNA segment is hybridized to a longer DNA strand (mimicking the binding of a random primer to the minus-strand DNA), this enzyme binds almost exclusively in an RNase H cleavage mode, and the active site of the polymerase domain does not bind to the 3' end of the RNA to extend this sequence. But if the RNA is the PPT primer, a portion of the reverse transcriptase binds to it in a polymerase-competent orientation, with the 3' end of PPT available for extension by the polymerase active site (Fig. 2).

Abbondanzieri and colleagues also show that, in the presence of dNTPs, the number of reverse transcriptase molecules that bind to the PPT primer in the polymerase-competent orientation increases. Moreover, if a short DNA primer is bound to the longer DNA template, most reverse transcriptase enzymes bind in the polymerase-competent orientation. These results indicate that the PPT sequence 'design' is such that not only does it avoid degradation, but it is also an efficient primer for the initiation of second-strand DNA synthesis.

Remarkably, HIV reverse transcriptase can 'flip' its orientation on a nucleic-acid substrate without dissociating from it⁵. Such a transition between two binding orientations is unexpected, given the extensive contacts between this enzyme and its nucleic-acid substrate. The authors also find that an incoming dNTP substrate can bind to a reverse transcriptase in complex with a template–primer duplex (which lacks a hydroxyl group in

the 3' end of the primer to prevent dNTP incorporation), thereby stabilizing the polymerase-competent orientation of reverse transcriptase. Formation of such a 'dead-end' complex has previously been reported⁵ to prevent a reaction that unblocks chain-terminated primers and to increase the efficacy of some chain-terminating drugs that target HIV reverse transcriptase.

The authors have made another intriguing observation: the drug nevirapine — an inhibitor of HIV reverse transcriptase that binds in a pocket at the base of the flexible 'thumb' sub-domain of the enzyme (Fig. 2a) — allows the enzyme to flip more rapidly. Nevirapine binding forces the thumb subdomain to undergo a conformational change that Abbondanzieri *et al.* propose relaxes the enzyme's grip on the nucleic-acid substrate, thus allowing it to switch orientations more rapidly. This observation implies that certain steps in the process of reverse transcription may be particularly susceptible to antiviral treatments — an idea that has also been proposed previously^{6,7}.

Abbondanzieri and colleagues' work vividly illustrates the importance of understanding how the orientational dynamics of reverse transcriptase relate to the enzyme's activities. Their findings also raise the question of whether similar surprises are in store with regard to the enzyme's interactions with the host tRNA, during the initiation of minus-strand-DNA synthesis. Another question is whether other multifunctional enzymes indulge in similar molecular acrobatics. Examples of such enzymes are DNA polymerases, which, like reverse transcriptases, have separate non-polymerase activities, or enzymes that interact with nucleic acids, such as helicases, ligases, topoisomerases, integrases and restriction endonucleases. Whatever the answers, this work suggests that reverse transcriptase is even more agile in its handling of nucleic acids than we thought. ■

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