

## Hands-on Activity *HIV Reverse Transcription and AZT*

hhmi BioInteractive

Student Handout

### HIV REVERSE TRANSCRIPTION AND AZT

#### INTRODUCTION

The human immunodeficiency virus (HIV) infects and destroys cells of the immune system, weakening a person's ability to fight other infections and diseases. Acquired immunodeficiency syndrome (AIDS) occurs when a person's immune system is severely compromised. Without treatment, most people with AIDS die. Fortunately, researchers have developed drugs to treat HIV infection.

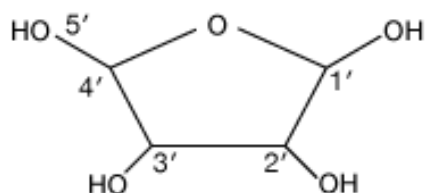
A combination therapy, or drug cocktail, of several drugs at one time makes an HIV infection a treatable chronic disease rather than a terminal illness. The drug azidothymidine (**AZT**) is one of the drugs commonly used in this drug cocktail. AZT targets a critical step in the HIV replication cycle: reverse transcription.

HIV has a single-stranded **RNA genome**. During reverse transcription an HIV enzyme converts the HIV RNA into DNA so that it can be inserted, or integrated, into the host cell genome. Reverse transcription is different from usual transcription, where DNA is transcribed to RNA. In this activity, you will learn how reverse transcription and AZT work. First, however, let's review the structures of DNA and RNA.

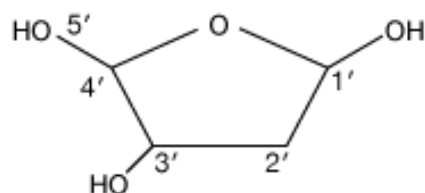
#### DNA and RNA Review

You probably know that the instructions for building all the proteins that make up an animal or plant cell are stored in DNA. DNA is a nucleic acid. RNA is also a nucleic acid. The genome of HIV is made of single-stranded RNA.

Nucleic acids (DNA and RNA) are made up of a sequence of **nucleotides**. All nucleotides have a common structure: a phosphate group linked to a sugar molecule (ribose in RNA and deoxyribose in DNA, **Figure 1**) that is in turn linked to one of four nitrogenous **bases** (**Figure 2**). The bases **adenine**, **guanine**, and **cytosine** are found in both DNA and RNA; **thymine** is found only in DNA, and **uracil** is found only in RNA. The bases are often abbreviated A, G, C, T, and U, respectively.

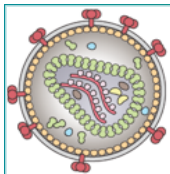


Ribose

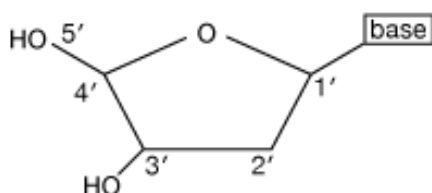


2-deoxyribose

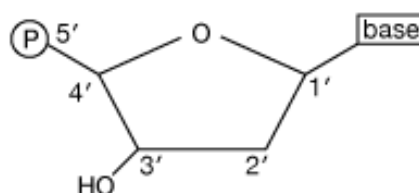
**Figure 1. The sugars of nucleic acids.** All nucleic acids contain a five-carbon sugar molecule. In RNA the sugar molecule is ribose and in DNA it is deoxyribose (the second carbon has a hydroxyl group and a hydrogen in ribose and two hydrogens in deoxyribose). By convention, the carbon atoms of the sugar are numbered with primes.



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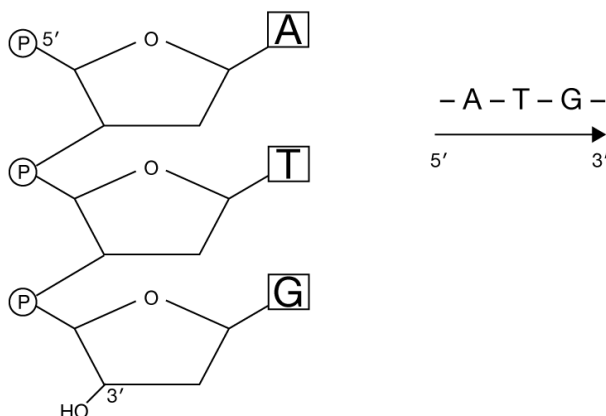
Nucleoside



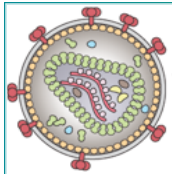
Nucleotide

**Figure 2. Nucleosides and nucleotides—what's the difference?** A sugar molecule bound to a base is called a nucleoside. The nucleosides are thymidine, adenosine, guanosine, cytidine, and uridine. When the nucleoside is bound to a phosphate group, it is called a nucleotide.

During RNA or DNA synthesis, the hydroxyl group on the 3' carbon of the sugar of one nucleotide forms a bond with the phosphate of another nucleotide. In this way, one nucleotide is joined to another to form a chain that lengthens in the 5' (phosphate) to 3' (hydroxyl) direction (**Figure 3**).

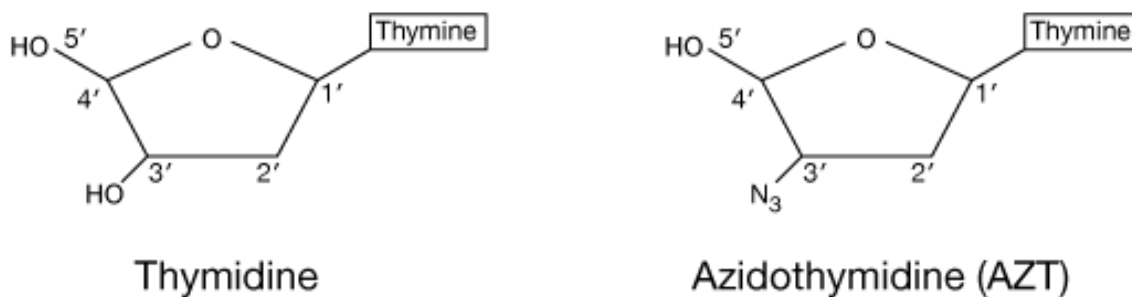


**Figure 3. Two illustrations of DNA nucleotide chains.** The left side of the figure shows the chemical structure of a DNA sequence that is three nucleotides long. The 5' end has a free phosphate group (attached to the 5' carbon of the ribose), and the 3' end has a free hydroxyl (OH) group. When a new nucleotide is attached, its phosphate group would form a bond with the 3' hydroxyl group, so the sequence grows in the 5' to 3' direction. The right side of the figure shows a simplified way of illustrating the same sequence. Although, strictly speaking, the letters A, G, C, T, and U stand for bases, they are also often used in diagrams to represent the whole nucleotides containing these bases. By convention, DNA sequences are written in the 5' to 3' direction.



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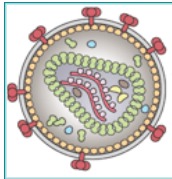
AZT is structurally very similar to the nucleoside thymidine (**Figure 4**). In a cell, AZT can become linked to a phosphate group to form a nucleotide that can be incorporated in a growing nucleic acid chain.



**Figure 4. AZT is structurally similar to thymidine.** Thymidine is a naturally occurring nucleoside. AZT is a drug.

HIV contains an enzyme called reverse transcriptase that transcribes RNA into DNA. When HIV infects a cell, the reverse transcriptase transcribes the viral RNA genome into a complementary DNA strand. The enzyme then catalyzes the synthesis of a second DNA strand, complementary to the first. The double-stranded viral DNA is then inserted into a host cell's chromosome. The host cell's RNA polymerase transcribes the viral DNA into RNA molecules that can serve both to provide instructions for the synthesis of viral proteins and as genomes for new viruses.

Let's examine this process!



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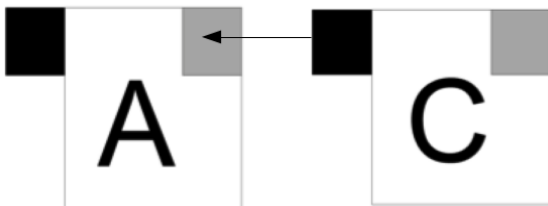
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### BACKGROUND QUESTIONS

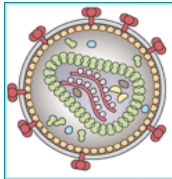
1. Draw a sequence of **10 RNA nucleotides** to represent a segment of the HIV genome. You can just use the shorthand version with a string of letters. Make sure you use each of the four RNA nucleotides at least once.
2. The first step in reverse transcription is the synthesis of a complementary DNA strand. Draw a diagram of the RNA segment from step 1 paired with DNA nucleotide bases.
3. The second step is the synthesis of a DNA strand complementary to the first to produce double-stranded viral DNA. Draw a diagram of a double-stranded DNA molecule.
4. Compare your drawing of the RNA/DNA nucleotide base pairs (in Question 2) to the DNA nucleotide base pairs (in Question 3), and in each drawing, circle the nucleotide base pairs that are different.
5. The double-stranded DNA is incorporated into the host cell DNA. What is the function of DNA in cells?

### ACTIVITY

Your teacher will provide an actual sequence of HIV RNA (written as a series of letters) and a set of **DNA nucleotides**. Using the nucleotides, synthesize a DNA strand complementary to the viral RNA template. The nucleotides should be assembled as follows (**Figure 5**).



**Figure 5.** Example of how the growing DNA chain is assembled. Each nucleotide cutout has a tab that sticks out which represents the 5' phosphate group that attaches to a sticky square that represents the 3' OH group.



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6. Record the sequence of the newly synthesized DNA strand below and then take apart your DNA sequence.
  
7. Now replace one of your T nucleotides with AZT. AZT is similar in molecular structure to thymidine but with one important difference. Examine Figure 3 showing the chemical structures of thymidine and of AZT. What are the differences between the two structures?
  
8. In cells where AZT is present, AZT can be incorporated in a growing DNA chain in place of a thymidine base. Predict what might happen if AZT is incorporated.
  
9. Repeat step 6 above in a cell where AZT is present. Write down the sequence of the DNA you constructed.
  
10. How is this sequence different from the sequence in step 6?
  
11. Using the information above, explain how AZT affects the process of reverse transcription.