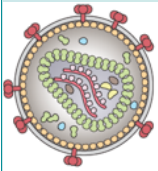


HIV REVERSE TRANSCRIPTION AND AZT

OVERVIEW

This hands-on activity is part of a series of activities and demonstrations focusing on various aspects of the human immunodeficiency virus (HIV) life cycle. In this activity, students will model how the anti-HIV drug AZT (azidothymidine) interferes with the process of viral replication and reduces the amount of virus in the body.

Students will first model reverse transcription, the process that results in the production of a double-stranded DNA copy of the HIV **single-stranded RNA genome**. (See introduction in the student document for a review of the process.) Using an actual HIV RNA sequence as a template, students will model the synthesis of a complementary strand of DNA by attaching nucleotides to one another. Then, students will demonstrate AZT's effect on this process. They will substitute AZT in place of thymidine. AZT lacks a hydroxyl (OH) group that is necessary for subsequent nucleotides to attach. When AZT is incorporated in a growing DNA sequence it prevents further nucleotides from being added, thereby blocking the production of HIV DNA.

Following this activity, it may be helpful for students to complete the HIV integration activity.

LEARNING OBJECTIVES

Students will be able to:

- Code DNA from RNA.
- Demonstrate the process of reverse transcription.
- Understand the role of the enzyme reverse transcriptase in the formation of viral DNA.
- Review the chemical structures of nucleic acids and identify structural similarities and differences between AZT and thymidine.
- Model how AZT inhibits DNA synthesis.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (2013)	HS-LS1-1; HS LS3-1; HS-LS3-2
AP Biology (2013)	3.A.1; 3.C.3
IB Biology	6.3

KEY TERMS

HIV, RNA, DNA, reverse transcription, reverse transcriptase, bases A, G, C, T, U (adenine, guanine, cytosine, thymine, and uracil), replication, nucleotide, nucleoside

TIME REQUIREMENTS

This lesson is designed to be completed in a 50-minute class period.

SUGGESTED AUDIENCE

This lesson is appropriate for high school Honors, AP, and IB, and Introductory Biology college students.

PRIOR KNOWLEDGE

Students should have studied DNA replication and transcription. They should know that DNA and RNA are made of nucleotides and have some familiarity with the chemical structures of nucleotides. Knowledge of the atomic structure of each nucleoside and their connection via phosphodiester bonds between the 5'-phosphate and 3'-hydroxyl functional groups is not required but will be helpful to more deeply understand how AZT interferes with DNA synthesis. The introduction to the student document contains information about the structure of nucleic acids and the difference between nucleosides and nucleotides.

MATERIALS

- Copies of the RNA template, which can be laminated to preserve them for future use. (The RNA template is available as a separate document on BioInteractive.)
- Copies of the DNA nucleotides and AZT, which can be laminated to preserve them for future use. (The nucleotides are available as a separate document on BioInteractive.)
- Optional Velcro tabs 0.9 cm (3/8 in.) size or slightly larger

For each group of two or three students, you will need 1 copy of the RNA template, 7 cytosines, 6 guanines, 3 adenines, 2 thymines, and 1 AZT.

TEACHING TIPS

Background Information

- An animation of the HIV life cycle is available at http://www.hhmi.org/biointeractive/hiv-life-cycle. The first 19 seconds of this resource describes the structure of HIV, including the RNA genome and reverse transcriptase enzyme. It then shows how HIV infects helper T cells (00:20 seconds through 1:25 minutes) and demonstrates reverse transcription, integration, viral protein synthesis, reproduction, and budding as steps in the HIV life cycle.
- Azidothymidine (AZT) was the first FDA-approved drug to treat HIV infection. AZT is a reverse transcriptase inhibitor that is structurally similar to the nucleoside thymidine. The 3' hydroxyl group of thymidine is replaced with an N_3 azide group, preventing bonding between nucleotides

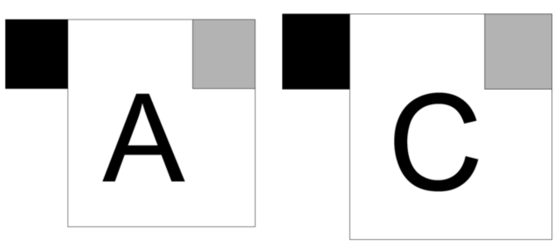
during the process of reverse transcription. The structure of AZT causes it to block further DNA synthesis when it is incorporated in a DNA strand.

- The introduction to the student document contains information about the structure of nucleic acids. Spend some time reviewing this information. You could assign it as a reading before doing the assignment in class and then spend some time reviewing the concept.

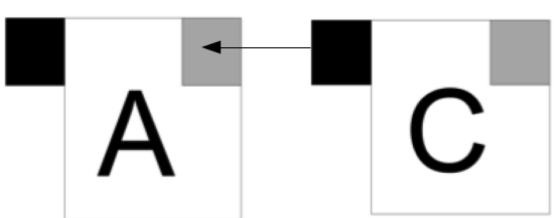
Preparing the RNA Template and Nucleotides

- Before class, prepare both the RNA template and the DNA nucleotides for this activity.
    - The RNA template is a single strand of nucleotides (GGGCCUGUUGCACCAGGC). This sequence is an actual HIV sequence.
    - The DNA nucleotide bases are individually cut. You may wish to laminate them and attach either Velcro® or some type of adhesive to the tabs.
    - If you use Velcro®, the "hook" of the Velcro® is always on the top right gray inset tab of the nucleotide base (facing up); this represents the 3' hydroxyl group. The "loop" of the Velcro® is always on the top left tab (facing down on the underside of the tab). This represents the 5' phosphate; see **Figure 1**.
    - The tabs should attach when the nucleotide bases bond together. The Velcro® or adhesives model the phosphate backbone of a nucleotide sequence. The edges of the nucleotides should touch and the bases align with those of the HIV RNA sequence.

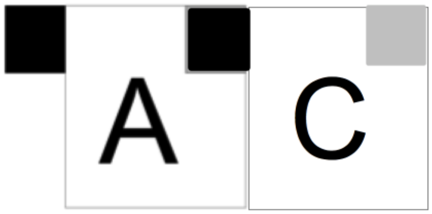
Figure 1. Steps in preparing the nucleotides.



Step 1: Cut out the laminated nucleotides and attach the Velcro to the tabs. For the black tabs, the Velcro goes on the underside of the tab and for the gray tabs the Velcro goes on top.



Step 2: Following the RNA template, attach the appropriate nucleotides to one another with the black tabs going on top of the gray tabs.



Step 3: Be sure that the edges of the nucleotides are touching. Each nucleotide should align with the one it is pairing with on the RNA template.

Conducting the Activity

- Depending on the size of the class, divide students into groups of 2, 3, or more. Give each group the RNA template and the DNA nucleotide bases. Tell students they will model how the enzyme reverse transcriptase builds a DNA sequence from the viral RNA template.
- Explain that in this activity they are building a single-stranded DNA sequence. In reality, the reverse transcriptase will also synthesize a complementary DNA strand and the double-stranded DNA will integrate into the host DNA genome.
- After students build the DNA sequence complementary to the viral RNA, ask them to take their sequences apart. Then, hand each group an AZT cutout (without a top-right Velcro tab) and take away one of their T nucleotides.
- Ask students to build a DNA sequence from the viral RNA, but this time have the enzyme reverse transcriptase introduce AZT into the DNA strand. They should substitute AZT at the one of the T locations and see that they cannot attach more nucleotides, meaning HIV replication is halted.
- The AZT can be incorporated anywhere in the sequence where a T would normally go. It does not have to go in the first position.

Wrapping up

- Once students complete the activity, you may want to show them an animation of how AZT blocks reverse transcriptase (http://www.hhmi.org/biointeractive/azt-blocks-reverse-transcriptase).
- The last part of the animation above deals with drug resistance. To learn more about drug resistance and why researchers give multiple drugs, watch a 3-minute clip from Lecture 3, *"Drugs and HIV Evolution," Chapters 10 and 11*. Open the lecture at https://www.hhmi.org/biointeractive/drugs-and-hiv-evolution and then click on Chapter 10 and watch from time 11:41 to 15:00.
- AZT is a nucleoside reverse transcriptase inhibitor (NRTI). A number of drugs called non-nucleoside reverse transcriptase inhibitors (NNRTIs) block the same step in the HIV life cycle as

NRTIs (reverse transcription) but by a completely different mechanism. Rather than being incorporated into the viral DNA like NRTIs, NNRTIs bind directly to the HIV reverse transcriptase enzyme, inhibiting its activity.

- As time allows, show students a short (1:50 sec) interview with an HIV-positive woman able to lead a normal life thanks to antiviral therapy. It is available at http://www.hhmi.org/biointeractive/benefits-antiretroviral-regimen

ANSWER KEY

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 include each of the four RNA nucleotides at least once.

Sequences will of course vary. The key is that students should show a single sequence that has the four nucleotides AUGC but not T.

Example: -U-C-C-C-U-U-C-A-G-A-

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.

Students should draw the same sequence as in the answer above and a second complementary sequence. Pairing should show U/A (U in the RNA strand and A in the DNA complement), G/C, C/G, A/T.

Example: -U-C-C-C-U-U-C-A-G-A-
-A-G-G-G-A-A-G-T-C-T-

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.

Now students should draw the same sequence as the DNA sequence in answer 2 and a complementary DNA sequence. Pairings should always be A/T and G/C.

Example: -A-G-G-G-A-A-G-T-C-T-
-T-C-C-C-T-T-C-A-G-A-

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.

Students should circle the U/A pairings in Question 2 and the T/A pairings in Question 3.

5. The double-stranded DNA is incorporated in the host cell DNA. What is the function of DNA in cells?

Students should state that DNA contains the genetic information of the cell and carries instructions for making proteins.

6. Record the sequence of the newly synthesized DNA strand below and then take apart your DNA sequence.

CCCGGACAACGTGGTCCG

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?

AZT's structure is very similar to that of thymidine except that it has N_3 instead of OH on the ribose.

8. In cells where AZT is present, AZT can be incorporated in a growing DNA chain in place of the thymidine base. Predict what might happen if AZT is incorporated.

Students should predict that reverse transcription will stop because the N_3 group cannot form a bond with the phosphate of another nucleotide.

9. Repeat step 6 above in a cell where AZT is present. Write down the sequence of the DNA you constructed.

CCCGGACAACG(AZT)

CCCGGACAACGTGG(AZT)

10. How is this sequence different from the sequence in step 1?

Students should say it is shorter.

11. Using the information above, explain how and why AZT affects the process of reverse transcription.

AZT's structure is very similar to that of thymidine except that it has N_3 instead of OH. The absence of the OH group prevents the formation of a bond between AZT and the next nucleotide in the sequence, thereby halting DNA synthesis and HIV reproduction.

AUTHORS

Written by Patricia Nolan Bertino, Scotia-Glenville High School, and Anthony Bertino, Canandaigua Academy. James Blankenship, PhD, Cornell University, advised on the project. Additional writing by Mary Colvard. Illustrations in student document by Chris Vargas.

Edited by Leah M. Cataldo, PhD, Buckingham Browne & Nichols School; Karen Gulliver, consultant; and Laura Bonetta, PhD, HHMI. Reviewed by Melissa Csikari, HHMI.

Scientific review by Allen Bateman, PhD, Debby Walser-Kuntz, PhD, Carleton College, Munir Syed, PhD, Hartwick College.