

Demonstration *HIV Protease Inhibitors*

HIV PROTEASE INHIBITORS

OVERVIEW

This demonstration is part of a series of activities and demonstrations focusing on various aspects of the human immunodeficiency virus (HIV) life cycle.

HIV is a retrovirus, a type of virus that integrates its genome into the host cell's genome. (Two other activities focus on the reverse transcription and integration steps of the viral replication cycle.) HIV has genes common to all retroviruses (the genes *gag*, *pol*, and *env*) that encode structural proteins and enzymes, as well as genes that are unique to its viral structure and function. This activity focuses on the *gag* and *pol* genes. These two genes are transcribed into a single RNA molecule, which is then translated to produce a single polyprotein. The Gag-Pol polyprotein is cleaved by the HIV protease enzyme to generate six proteins essential for assembling the virus particle.

During this activity, you will demonstrate the mechanism by which the Gag-Pol polyprotein is produced and cleaved by HIV protease. The demonstration models this process and also shows how inhibiting HIV protease activity prevents the virus from creating mature proteins. Protease inhibitors are a class of drugs used to treat HIV infection.

KEY CONCEPTS AND LEARNING OBJECTIVES

- HIV RNA can be translated into polyproteins; post-translational cleavage of these polyproteins results in the generation of functional viral proteins.
- One of the drugs developed against HIV interferes with the function of an HIV enzyme, a protease, responsible for cleaving HIV polyproteins.

Students will be able to

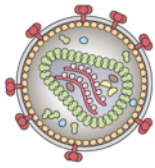
- apply the concepts of protein synthesis and enzyme function to viral replication and the HIV life cycle.

CURRICULUM CONNECTIONS

| Curriculum | Standards |
|-------------------|----------------------------|
| NGSS (2013) | HS-LS1-1 |
| AP Biology (2013) | 3.B.1; 3.C.3; 4.A.1; 4.B.1 |

KEY TERMS

virus, genome, DNA, mRNA, gene, transcription, translation, enzyme, ribosome, inhibitor, protease, protein, capsid, reverse transcriptase, integrase, polymerase, 5' cap, 3' tail



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TIME REQUIREMENTS

If students are familiar with the HIV life cycle, the demonstration itself takes approximately 15 minutes. Preparation of materials should take about 30 minutes the first time it is done.

SUGGESTED AUDIENCE

This lesson is appropriate for AP Biology.

PRIOR KNOWLEDGE

Students should be familiar with the processes of transcription and translation in eukaryotic cells.

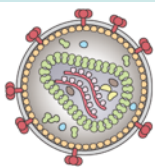
MATERIALS

- Wooden dowel 7/8 inch in diameter to represent HIV RNA.
 - The dowel can be purchased at most home improvement or craft stores or online.
 - The dowel should be prepared ahead of class by coloring different parts to indicate different genes or groups of genes.
- Roll of cash register tape 2 ¼" × 130 ft (57 mm × 39 m) to represent the host cell's ribosome.
 - The cash register tape is available at most business supply stores or online.
 - The paper rolled out of the cash register tape will represent the polyprotein being synthesized.
 - Be sure that the central core of the register tape fits over the wooden dowel.
- Scissors to represent the viral protease enzyme.
- Styrofoam cone 2 ¾" × 1 7/8" (69.85 mm × 47.62 mm) to represent the protease inhibitor.
 - The Styrofoam cones can be found in the flower arrangement department of most craft stores or online.
 - The cone should be large enough to fit between the blades of the scissors and prevent the scissors from cutting.

TEACHING TIPS

Before conducting the demonstration

- If students are not familiar with the structure of HIV, it will be helpful for them to become familiar with the basics. HIV is an enveloped retrovirus that consists of an RNA genome, a viral capsid, and an outer membrane, or envelope, with embedded proteins. To see the structure of HIV, visit the interactive "Virus Explorer" at <https://www.hhmi.org/biointeractive/virus-explorer>. Show students the HIV cross section and point out to them the HIV capsid protein (encoded by the *gag* gene) and the integrase, reverse transcriptase, and protease enzymes (encoded by the *pol* gene). Also point



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out the HIV RNA genome. Note that the full HIV genome is encoded on a single strand of RNA. However, each virus particle contains two separate, identical RNA strands.

- Students should also be familiar with the key events in the HIV life cycle. Mainly, when HIV infects a cell, the HIV RNA genome is reverse-transcribed into DNA, which is then integrated into the human genome. This DNA copy of the HIV genome is then transcribed into RNA by the host cell machinery. The HIV RNA can be incorporated into new virus particles or translated into proteins. An animation of the HIV life cycle is available at <https://www.hhmi.org/biointeractive/hiv-life-cycle>.
- Students may wonder whether human cells also produce polyproteins. Human cells do not produce polyproteins. In human cells, mRNAs are typically translated into single proteins. However, several RNA viruses produce polyproteins. Because this mechanism is not used by host cells and utilizes viral enzymes, these enzymes make good targets for antiviral drugs.

After completing the demonstration

- After completing the activity, you may want to show students an animation demonstrating protease inhibition available at <http://www.hhmi.org/biointeractive/protease-inhibitors>.
- Students may wonder how protease inhibitors relate to other drugs used to treat HIV. Other drugs are available that target different steps in the HIV life cycle: viral entry, reverse transcription, integration, and protein cleavage. The first such drug, azidothymidine, which inhibits the viral reverse transcriptase enzyme, was approved by the Food and Drug Administration in 1987. The first protease inhibitor, saquinavir, was approved in 1995. As of 2015, eight protease inhibitors were commercially available to treat HIV. Because HIV mutates rapidly due to the lack of proofreading capacity of reverse transcriptase, resistance can easily develop against any drug. To combat resistance, doctors typically give patients several different antiretroviral drugs. This combination treatment lowers the chances of the virus becoming resistant because the virus must become resistant to multiple drugs that target different aspects of its life cycle.
- Although human cells do not produce polyproteins, they do produce proteases. Proteases modify proteins once they are made. Many proteins that are exported from the cytoplasm are synthesized by ribosomes as proproteins (or preproteins) located on the rough endoplasmic reticulum and subsequently cleaved by proteases to become functional proteins. For example, proteases activate proproteins such as proinsulin and blood clotting factors. Proteases are also responsible for destroying proteins once they are no longer needed.

PROCEDURE

Background Information

The HIV genome contains nine genes. Three of them (*gag*, *pol*, and *env*) are common to all retroviruses and the others are unique to HIV (see **Figure 1** for a simplified schematic). In this activity, we will focus on the *gag* and *pol* genes. The *gag* gene encodes three proteins that comprise the viral capsid. The *pol* gene encodes three enzymes that are essential for viral genome replication and integration into the host genome (reverse transcriptase, protease, and integrase). When HIV DNA is integrated into the human genome, it is

transcribed to produce a primary RNA that can either become part of new viruses or be translated into various different proteins. One of the proteins that are generated is a Gag-Pol polyprotein.

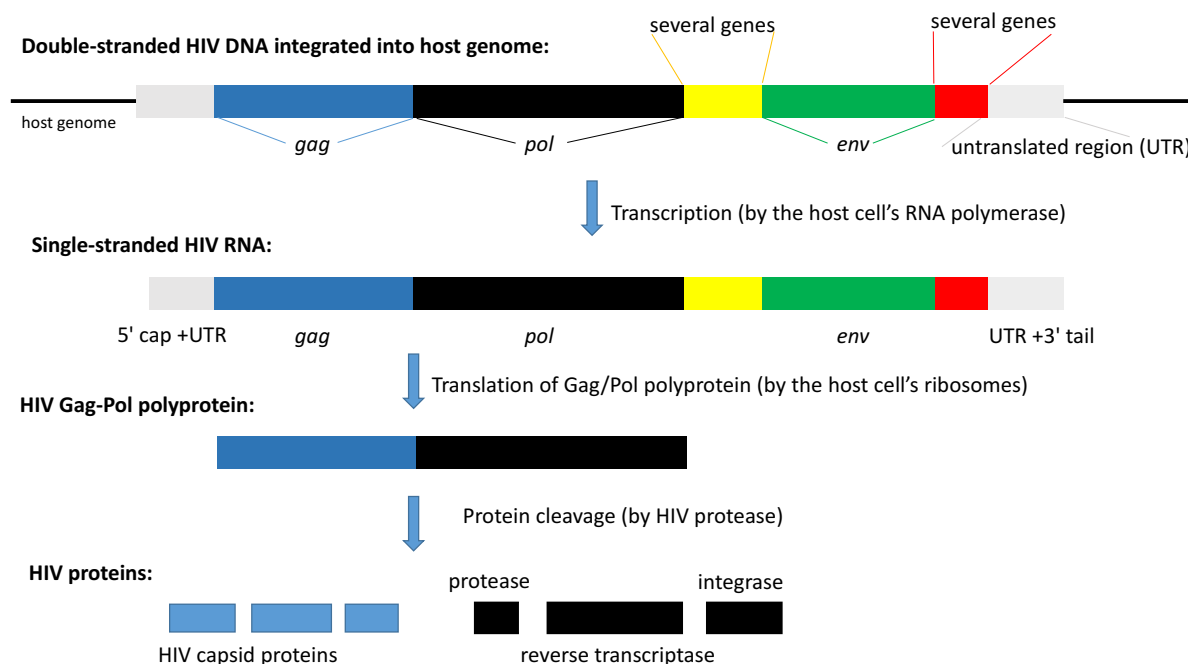


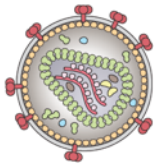
Figure 1. Simplified illustration of the HIV genome and the production of the Gag-Pol polyprotein. The HIV DNA integrated into the host genome is represented by the colored rectangles and the host genome is the thinner line. The HIV DNA contains several genes that encode structural and regulatory proteins and enzymes. This illustration shows the three main genes—*gag*, *pol*, and *env*—that are conserved among all retroviruses. The coding region of the virus is flanked by noncoding regions called long terminal regions (or LTRs) that contain regulatory elements for transcription. The HIV DNA is transcribed into a single RNA molecule that is about 9,700 nucleotides long and that can be translated to produce a peptide Gag-Pol polyprotein. HIV protease then cleaves the Gag-Pol polyprotein to produce the proteins that make up the HIV capsid and the enzymes protease, reverse transcriptase, and integrase. (The HIV RNA can also be spliced to produce several different RNAs that can then be translated into various other proteins, including regulatory proteins and envelope proteins. However, this activity only focuses on production of the Gag-Pol polyprotein.) The 5' cap and 3' tail are components common to all eukaryotic mRNAs. The 5' cap protects the mRNA from degradation and allows the cell's ribosome to bind to the mRNA to start translation. The 3' tail also protects the mRNA from degradation.

Preparing the dowel

The dowel represents HIV RNA. Using different color markers or paint, color in an area on the dowel for each of the key protein regions (Figure 2).



Figure 2. Example of what the dowel should look like.



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Table 1. Suggested sections for marking the RNA dowel.

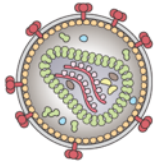
| Region | Genes | Color | Approximate length on dowel (mm) |
|-----------------|---------------------------|----------|----------------------------------|
| 5' cap and UTR | | no color | 60 |
| | <i>gag</i> | blue | 220 |
| | <i>pol</i> | black | 310 |
| | <i>vf/vpr/tat/rev/vpu</i> | yellow | 100 |
| | <i>env</i> | green | 200 |
| | <i>tat/rev/nef</i> | red | 30 |
| UTR and 3' tail | | no color | 60 |

Note: You can pick any colors; these are just suggestions. The genes *vf/vpr/tat/rev/vpu* receive a single color for simplicity. The same is true for *tat/rev/nef*. UTR stands for untranslated region; this is a portion of the RNA that is not translated into protein. The 5' cap and 3' tail are components common to all eukaryotic mRNAs. The length of different colored segments on the dowel roughly correspond to the lengths of genes.

Conducting the demonstration

Part 1. Modeling Gag-Pol Polyprotein Synthesis

1. Show students the dowel and explain that it represents HIV RNA that was transcribed by the viral DNA integrated into the host genome. Explain that each color represents a different gene or cluster of genes. Point out the *gag* and *pol* genes (blue and black color in Figure 2). These two genes can be translated as a single polyprotein. The polyprotein is then cleaved by the HIV protease into several proteins. You will model this process.
2. Slide the roll of cash register tape onto the dowel. Explain that the roll represents the ribosome. The ribosome recognizes the 5' cap sequence of the mRNA and starts translation.
3. Have one or two student volunteers hold the dowel, one at each end.
4. Ask another volunteer to slowly move the roll of cash register tape along the dowel. Start unrolling the tape as you move the ribosome over the start of the *gag* gene (the blue section).
5. As the register tape (ribosome) moves along the dowel (RNA), it “translates” the polyprotein, which is represented by unrolling the paper (Figure 3).



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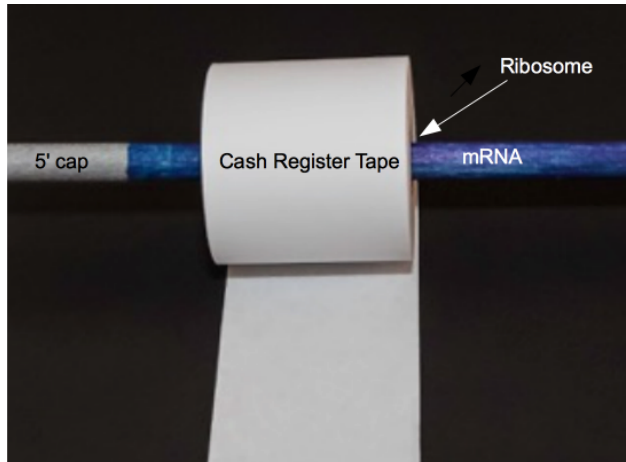


Figure 3. Modeling the translation of HIV RNA. The dowel represents mRNA and the cash register tape represents the ribosome. Start translation (which means unrolling the tape) at the beginning of the *gag* gene, which is marked in blue here. The paper coming off the register tape represents the Gag-Pol polyprotein.

6. When the roll of tape reaches the end of the *gag* gene, stop and mark the end of the Gag protein on the paper (Figure 4).
7. Continue to unroll the tape until you reach the end of the *pol* gene (black section). As you reach the end, rip the paper to end translation.

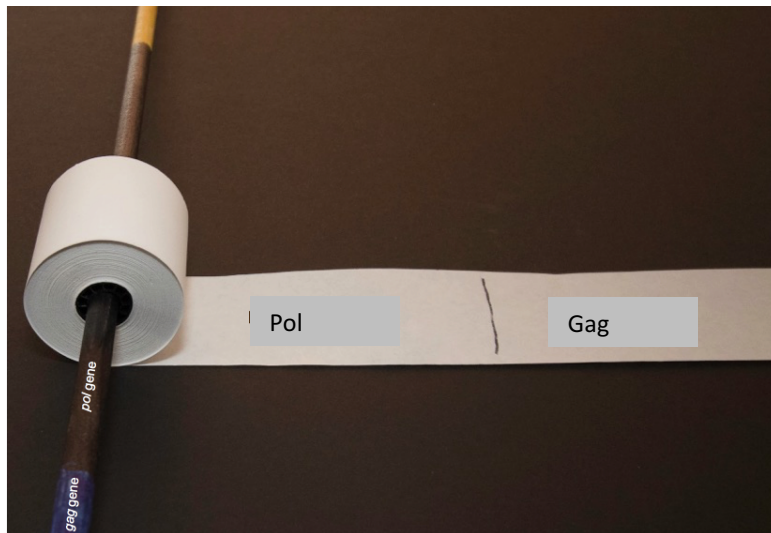
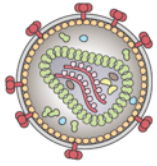


Figure 4. Continuing to translate the Gag-Pol polyprotein. Mark the end of the *gag* gene with a line on the white roll of paper and continue to translate the HIV mRNA until you reach the end of the *pol* gene (colored black here).

8. The white paper you produced represents the Gag-Pol polyprotein.

Part 2: Modeling cleavage of the Gag-Pol polyprotein (protease activity)

9. After the Gag-Pol polyprotein has been translated, the HIV protease (scissors) cuts the polyprotein into separate proteins to generate mature proteins that make up new viral particles (Figure 5). The *gag* gene encodes the proteins that make up the capsid, the protein coating around the HIV viral genome. The *pol* gene encodes three enzymes: protease, reverse transcriptase, and integrase.



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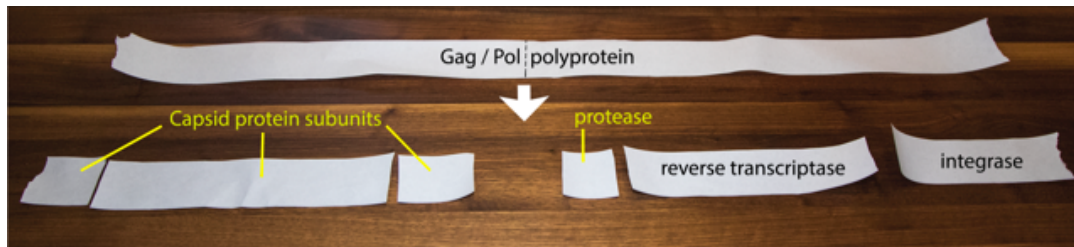


Figure 5. Modeling the cleavage of the Gag-Pol polyprotein into six proteins. The first three proteins, encoded by the *gag* gene, are part of the HIV capsid. The other three, encoded by the *pol* gene, are the HIV enzymes protease, reverse transcriptase, and integrase.

Part 3: Modeling the protease inhibitor

To demonstrate the action of a protease inhibitor, repeat steps 1 through 8 above. Then, before step 9, wedge a Styrofoam cone between the blades of the scissors (Figure 6). This action models how an inhibitory molecule may bind the active site of protease enzyme and prevent it from binding to its target (in this case, the register tape representing the HIV polyprotein Gag-Pol). If the action of the protease is blocked, then the Gag-Pol polyprotein is not separated into individual proteins and cannot generate new viruses.



Figure 6. Modeling the action of the protease inhibitor. The scissors represent the protease enzyme, the blades of the scissors represent the active site, and the Styrofoam cone represents the protease inhibitor drug.

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