Grade: 12	**Subject:** University Preparation Biology	**Unit:** Molecular Genetics

Website-Therapeutic Proteins [https://therapeuticproteins.weebly.com]

Rationale:
Students will explore the processes of protein synthesis, post-translational modifications, DNA cloning, recombinant DNA and transformation by watching videos and answering associated questions. This information will help students understand the exciting and important research that the Wakarchuk laboratory at Ryerson University is undertaking in relation to therapeutic proteins.

Background Information:
Researchers in the Wakarchuk laboratory at Ryerson University are working with *E. coli* to engineer it to complete human-like glycosylation functions on human therapeutic proteins. Glycosylation is the most prevalent and diverse protein modification that occurs in humans, with 70% of human proteins being glycosylated. Glycosylation is the addition of a carbohydrate unit or chain to a protein. Sialylation is a type of glycosylation that involves the addition of sialic acid, a type of sugar, to proteins. The addition of sugars, and in particular sialic acid, to therapeutic proteins will reduce side effects and increase circulatory half-life of therapeutic proteins in patients.

After obtaining a greater understanding of glycosylation and its abundance in human proteins, along with its importance for therapeutic protein therapy, the desire to relate it to high school science concepts became a priority. Glycosylation is one of several types of post-translational protein modifications, but it is the most widespread. Protein synthesis is part of the molecular genetics unit of the Grade 12 University Preparation Biology course. This website allows for a greater understanding of the various types of post-translational modifications of proteins that are part of protein synthesis. In addition, the differences between prokaryotic and eukaryotic cells, why *E. coli* is used as a model organism, and various biotechnology techniques are reviewed. A page is devoted to the research taking place in the Wakarchuk laboratory at Ryerson University.

Curriculum Connections:
- D3. demonstrate an understanding of concepts related to molecular genetics, and how genetic modification is applied in industry and agriculture
- D2.1 use appropriate terminology related to molecular genetics, including, but not limited to: polymerase I, II, and III, DNA ligase, helicase, Okazaki fragment, mRNA, rRNA, tRNA, codon, anticodon, translation, transcription, and ribosome subunits
- D3.2 compare the structures and functions of RNA and DNA, and explain their roles in the process of protein synthesis
- D3.3 explain the steps involved in the process of protein synthesis and how genetic

expression is controlled in prokaryotes and eukaryotes by regulatory proteins (e.g., the role of operons in prokaryotic cells; the mechanism of gene expression in eukaryotic cells)
- D3.5 describe some examples of genetic modification, and explain how it is applied in industry and agriculture (e.g., the processes involved in cloning, or in the sequencing of DNA bases; the processes involved in the manipulation of genetic material and protein synthesis; the development and mechanisms of the polymerization chain reaction)
- D3.6 describe the functions of some of the cell components used in biotechnology (e.g., the roles of plasmids, restriction enzymes, recombinant DNA, and vectors in genetic engineering)

Lesson Objectives/Concepts:
- Students are responsible for learning several concepts on their own before they are taught/reviewed in class
- Students will review the difference between prokaryotic and eukaryotic cells
- Students will understand why *E. coli* is a model organism
- Students will learn about protein synthesis, post-translational modifications and biotechnology techniques
- Students will learn about the exciting and important research taking place in the Wakarchuk laboratory at Ryerson University around therapeutic proteins

Materials: Access to the Internet, student handout, chart paper, overhead projector, blackboard.	**Time:** 2 periods A period is 75 minutes.

Introduction (10 minutes):
Questions to ask the class:
1. What are the main differences between prokaryotic and eukaryotic cells?
2. Briefly describe protein synthesis in your own words.
3. What are protein modifications, and more specifically, post-translational modifications?
4. Describe DNA cloning, recombinant DNA and transformation in your own words.

The answers to these questions will give the teacher an idea of the readiness level of the class.

Activities/Procedure (120 minutes):
Pre-teaching, done in advance of this lesson:
- Ensure you have taught DNA structure and replication before asking students to visit this website.
- Ensure you have given students a couple of days to visit the website and answer the video associated questions.

Lesson:

- Students should be divided into small groups. Each group is responsible for one topic that the website covers, including the research occurring in the Wakarchuk laboratory. Depending on the size of the class, the division of topics will vary. Groups of students should work together to go over the topic they have been assigned until they are comfortable explaining it. Using chart paper, an overhead projector or the blackboard, each group is to teach the class their part. Depending on the division of topics, the amount of time allotted to each group will vary.
- Groups are to take up the questions associated with their topic (video) with the class.
- The teacher should circulate around the room guiding students as necessary, and answering any questions that come up as students prepare their lesson.

Summary/Closure (20 minutes):

- The teacher should review the key concepts that were learned from the website with the class.

Assessment:

- As you are circulating around the classroom, assess the level of understanding amongst the groups of students.
- You may collect student answers to video associated questions to assess them for accuracy.
- Answers to website video questions are provided.

Therapeutic Protein Website: Answers to Video Questions

Video 1: Introduction to Cells: The Grand Cell Tour

1. What are the 3 postulates of the Modern Cell Theory?
   - The cell is the smallest living unit in all organisms
   - All living things are made of cells (one or more)
   - All cells come from other pre-existing cells

2. What are the 2 prokaryotic kingdoms?
   - Archaea
   - Bacteria

3. What are the 4 eukaryotic kingdoms?
   - Plants
   - Animals
   - Fungi
   - Protists

4. What are 3 similarities between prokaryotic and eukaryotic cells?
   - Both have ribosomes, cytoplasm and a cell membrane

5. What are 2 differences between prokaryotic and eukaryotic cells?
   - Eukaryotic cells have membrane bound organelles while prokaryotic cells do not
   - Eukaryotic cells have their DNA in a nucleus while prokaryotic cells do not (in the nucleoid region)

6. What is the function of ribosomes?
   - To make proteins from codes (genes) found on DNA

Video 2: Protein Synthesis

<u>Transcription</u>

1. Where does it occur?
   - In the nucleus

2. What happens in this process?
   - mRNA is made (copied) from a DNA template (gene)

3. What enzyme is involved in this process?

- RNA polymerase

4. What are the complementary base pairs of DNA?
   - Adenine and Thymine
   - Guanine and Cytosine

5. When mRNA is made from a strand of DNA, what is the mRNA base complement to adenine on the DNA? Why?
   - Uracil
   - RNA does not have thymine

Translation

1. Where does it occur?
   - In the cytosol/cytoplasm

2. What 2 parts make up ribosomes?
   - Protein and RNA

3. What are the 3-base codes in mRNA called?
   - Codons

4. What is the purpose of tRNA?
   - To transfer the correct amino acid to the ribosome to build the protein/polypeptide

5. What is found on the two ends of tRNA?
   - One end has an anti-codon that matches a specific mRNA codon
   - One end has a specific amino acid

6. What is the start codon? What amino acid does it code for?
   - AUG
   - Methionine

7. What kind of bond links amino acids together?
   - Peptide bond

8. When does protein synthesis stop? Why?
   - It stops when a stop codon is reached that does not code for an amino acid

Video 3: Protein Modifications

1. What are the 2 categories of protein modifications?
   - Co-translational and post-translational

2. The video focuses on the category that most protein modifications fall under. Which category is it?
   - Post-translational

3. What is glycosylation and what type of proteins does it happen to?
   What is glycosylation important for?
   Provide an example.
   - The addition of carbohydrate to a protein that is embedded in the cell membrane
   - It is important for identifying cells
   - ABO blood groups

4. What is lipidation and what type of proteins does it happen to?
   Provide an example.
   - The addition of a lipid to a protein that is found attached to the cell membrane
   - GPI anchor that attaches or tethers proteins to the cell membrane

5. What is phosphorylation? Provide an example.
   - The addition of a phosphate group to a protein or enzyme
   - Sodium-potassium pump
6. What is methylation? Provide an example.
   - The addition of methyl groups to proteins
   - Histones are methylated proteins around which DNA wraps itself.

7. What is proteolysis? Provide an example.
   - The cutting of a protein to make it functional
   - Insulin

8. What is ubiquitination?
   - The addition of ubiquinone (protein) to another protein to mark the protein for degradation/breakdown

Video 4: DNA Cloning and Recombinant DNA

1. What is DNA cloning?
   - The creation of identical copies of a piece of DNA

2. What do we use to cut a gene of interest out of DNA?
   - Restriction enzymes

3. What is a plasmid?

- A circular piece of genetic material (DNA) that is found outside of the main chromosome of bacteria and can be replicated and expressed
    - Often carry antibiotic resistance genes

4. What does DNA ligase do?
    - It ligates (sticks) the DNA together by establishing phosphodiester bonds
        - We now have recombinant DNA (more than one source)
        - Ensures the plasmid DNA and gene of interest are one piece

5. What does heat shock help bacteria do?
    - It helps them take in the recombinant plasmid

6. Why is it useful to add an antibiotic resistance gene to a recombinant plasmid?
    - You can grow the bacteria in the presence of the antibiotic to determine which bacteria took in the plasmid (because they will live in its presence)

7. What is the name of the process that uses the steps described in this video to create multiple copies of a gene of interest in bacteria?
    - Transformation