Track that Protein: A Unit on Protein Trafficking for Advanced High School Biology Students

Jacqueline Katz
Princeton High School
Princeton, NJ 08540
jacquelinekatz@princetonk12.org

Mentored by:
Dr. Lisa Denzin
Child Health Institute of New Jersey
Rutgers, The State University of New Jersey
New Brunswick, NJ 08901

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Teacher's Section

Science Background

The synthesis of proteins in a cell is facilitated by **ribosomes**. While protein synthesis is occurring, **polypeptide** chains get trafficked to a sub-cellular location; localization is typically dictated by **signal sequences** in the primary structure. The ribosomes regularly dock on the **endoplasmic reticulum** and synthesize the polypeptide chain through the membrane of the ER. This allows for folding and modification of the polypeptide to occur. Once the protein is properly folded and completely synthesized it is moved from the ER to another subcellular compartment or out of the cell via **transfer vesicles**. If the protein is intended for export from the cell, it typically moves from the ER to the **Golgi apparatus** where further modifications take place. The proper trafficking of proteins is vital for functioning. Several diseases including, ALS, retinitis pigmentosa and Wilson disease have been associated with protein mis-localization within the cell.[1] The mis-localization that occurs in these diseases have been associated with mutations in signal sequences as well as improper folding and association with cargo proteins. Similarly, to these errors in the nervous and excretory system the immune system can suffer if important proteins are not properly localized.

The immune system is a complex combination of cells and organs that respond to **pathogen** invasion in order to prevent illness in the body. The **immune system** of humans contains both a non-specific immune response (**innate**) and a specific immune response (**adaptive**). The innate response occurs quickly and protects the body from many different pathogens by using a combination of physical barriers and phagocytic cells. The adaptive response is developed for a particular pathogen/**antigen** and is slower to begin. The development of this specific response depends greatly on the production of proteins inside **antigen presenting cells** and **lymphocytes**.

Major Histocompatibility Complex (MHC) proteins are human cell surface proteins vital for the presentation of antigen and initiation of an adaptive immune response. MHC proteins are divided into two classes based on their structure, **MHC Class I** and **MHC Class II**. The Class I receptor is recognized by **$CD8^+$ T cells** and allows for the initiation of the **cell-mediated immune response**. The class II receptor is recognized by **$CD4^+$ T cells** allowing for the subsequent activation of **B cells** and initiation of the **humoral immune response**. The pathways taken by these proteins inside the cell prior to surface presentation differs.

MHC Class I is produced in the endoplasmic reticulum and remains there to be loaded with antigen that gets processed in the **proteasome**. Once MHC Class I is loaded with antigen, it is sent through the Golgi apparatus to the cell membrane following the pathway of a typical secretory protein. MHC Class II is created in the endoplasmic reticulum and sent to the lysosome/endosome where it is loaded with antigen that has entered the cell via endocytosis. This difference in protein processing is largely due to the interaction between MHC Class II and another protein, **Invariant Chain**. The invariant chain prevents MHC Class II from being sent to the Golgi apparatus and directs trafficking to **endosomes/lysosomes**. The antigen loaded MHC class II is then sent to the cellular membrane from the endosome. It is possible that the cytoplasmic tail of the MHC molecules dictates the differences in pathway through the cell.

In scientific study it is possible to determine the localization of proteins within the cell by preforming **cellular fractionation**. Cell fractionation uses **differential centrifugation** to separate the organelles based on density. Cellular compartments with a high density (mitochondria, chloroplast, nucleus) can be settled out of solution with lower speeds of centrifugation, while less dense cellular compartments (lysosomes, endosomes) require high speed centrifugation. Verification of adequate fractionation can be confirmed using **western blotting** and organelle-specific marker proteins.

Western blotting is an analytical technique used to detect proteins in an extract or sample. In the case of cell fractionation, samples taken at different phases of the differential centrifugation process can be analyzed for proteins known to act in that compartment. For example, many of the electron transport chain proteins can act as positive identification for mitochondrial separation. In order to perform a western blot,

proteins from the extract must first be separated using **SDS-PAGE**. This process denatures proteins and separates them based on molecular mass. The less massive proteins are able to move through the polyacrylamide gel with less resistance than larger proteins. Therefore, when the process is complete the smaller proteins will be located closer to the bottom of the gel than the larger proteins. The movement of the proteins is propagated by an electric field; SDS is a negatively charged molecule that is attracted to the positive electrode.

****Key terminology has been bolded; it is advised that students have a general understanding of these concepts prior to performing the lab experiments.**

Student Outcomes

1. This unit provides advanced biology students an opportunity to expand on their understanding of protein synthesis and the convergence of many variables in order to ensure proper functioning. Students will also gain an understanding of the organization of the immune system and the vital part that antigen presentation plays in triggering an immune response.
2. Once students cover the concepts of transcription and translation, they can make the connection back to the organelles of the cell to understand where the newly synthesized proteins go. This unit will cover the concept of **protein signaling/trafficking**, **organelles involved in protein synthesis**, **organelle specialization**, **processes/components involved in an immune response, role of antigen presentation in the immune system, cellular fractionation**, and **western blotting.**
3. *NGSS Addressed*:
    a. HS-LS1-2 Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms
    b. Science and Engineering Practices:
        i. Developing and Using Models
        ii. Planning and Carrying out Investigations
        iii. Analyzing and Interpreting Data
        iv. Using Mathematics and Computational Thinking
        v. Constructing Explanations and Designing Solutions
        vi. Obtaining, Evaluating, and Communicating Information

Learning Objectives

Students will be able to:

1 Explain the possible modes of protein movement throughout the cell
2 Outline the processes involved in a specific immune response
3 Experimentally determine the movement of MHC Class I and Class II through the cell
4 Conduct a western blot assay and analysis

Time Requirements

The completion of this unit will take roughly 2 weeks.

Unit Component	Time Allotted
Protein Trafficking Exploration	3-45-minute periods
Understanding the Immune System PPT	1-45 minute period
Pre-lab Procedure Introduction	1-45-minute period
Run Gel	1-45-minute period
Transfer to Membrane	1-45-minute period
Primary antibody set-up	1-45-minute period
Secondary antibody set-up	1-45-minute period
Visualization	1-45-minute period
Analysis and Reflection	1-45-minute period
Optional MHC Class II Extension	1-45-minute period
NOTE: All incubations are set to happen outside of class and will need to be monitored by the teacher	

Advance Preparation

1 Buffer Preparation
   - *a.* SDS-PAGE Running Buffer = 900 ml MilliQH_2O and 100 ml 10 x SDS-PAGE Running Buffer (30.0 g Tris base, 144.0 g glycine, 10.0 g SDS in 1000 ml of H_2O)
   - *b.* Transfer Buffer (for 4 liters) = 56 g glycine, 12 g Tris base, 1 L methanol, 3 L MilliQH_2O
   - *c.* Blocking Buffer = 5% skim milk powder (store bought) in PBS-Tween and 0.02% sodium azide
   - *d.* Lysis Buffer = 2.375 mL 1x TBS (20mM TRIS pH 8/130 mM NaCl), 0.125 ml 20% Tx 100, ¼ Protease Inhibitor Tablet

2 Cells (cells will need to be grown, collected, and lysed in lysis buffer)
   - *a.* HeLa CIITA cells (HeLa cells with promotor for MHC class II)
     - *i.* 10 million cells can be lysed in 200 μl of lysis buffer
     - *ii.* Include cells with 200 μl of lysis buffer for 30 minutes on ice, vortex intermittently
     - *iii.* After 30 minutes spin cells at 14,000 rpm at 4°C for 5 minutes
     - *iv.* Transfer the cell lysate to a tube containing 50 μl of 5x reduced sample buffer
   - *b.* 3T3 cells (mouse fibroblasts)
     - *v.* Follow same procedure at HeLa CIITA cells
   - c. Aliquot the cellular lysates into micro-centrifuge tubes according to the table below.

Group 1 (Anti-Calreticulin)	
Total	20 µl HeLa CIITA
Endoplasmic Reticulum	20 µl HeLa CIITA
Golgi Apparatus	20 µl 3T3 cells
Membrane	20 µl 3T3 cells
Group 2 (Anti GM-130)	
Total	20 µl HeLa CIITA
Endoplasmic Reticulum	20 µl 3T3 cells
Golgi Apparatus	20 µl HeLa CIITA
Membrane	20 µl 3T3 cells
Group 3 (Anti-MHC I)	
Total	20 µl HeLa CIITA
Endoplasmic Reticulum	10 µl HeLa CIITA with 10 µl lysis buffer
Golgi Apparatus	5 µl HeLA CIITA with 15 µl lysis buffer
Membrane	20 µl HeLa CIITA
Group 4 (Anti-MHC II)	
Total	20 µl HeLa CIITA
Endoplasmic Reticulum	5 µl HeLa CIITA with 15 µl lysis buffer
Golgi Apparatus	20 µl 3T3 cells
Membrane	20 µl HeLa CIITA

3 Antibody dilutions (make all dilutions in blocking buffer)
   a. Anti-Calreticulin= 1:1000
   b. Anti-GM-130 = 1:400
   c. Anti-MHC I = 1:5,000
   d. Anti-MHC II = 1:5
   e. Goat-anti-Mouse IgG HRP = 1:2000

Materials and Equipment

1 5x Reduced Sample Buffer
2 Anti-Calreticulin (http://www.enzolifesciences.com/ADI-SPA-601/calreticulin-monoclonal-antibody-fmc-75/)
3 Anti-LAMP 1
4 Anti-GM130 (https://www.abcam.com/gm130-antibody-ab169276.html)
5 Anti-MHC I (XD5.A11 Ascities)
6 Anti- MHC II (3Bio.7 TCSN)
7 Goat-Anti-Mouse IgG HRP
8 SDS-PAGE pre-cast gel
9 SDS-PAGE gel box and power supply
10 SDS-PAGE transfer apparatus
11 Nitrocellulose Membrane (http://www.emdmillipore.com/US/en/product/Immobilon-P-PVDF-Membrane,MM_NF-IPVH00010)
12 Micropippettes with tips (2-20µl and 20-200µl)
13 Whatman Cellulose Filter paper (https://www.sigmaaldrich.com/labware/labware-products.html?TablePage=17207008)
14 Molecular Weight Marker (https://www.sigmaaldrich.com/life-science/proteomics/protein-electrophoresis/molecular-weight-markers.html#Prestained%20Blue%20Marker)
15 Amplified Opti-4CN Substrate Kit #1708238 (https://www.bio-rad.com/en-us/sku/1708238-amplified-opti-4cn-substrate-kit?ID=1708238#)

Student Prior Knowledge and Skills

1. Students should have a solid understanding of protein synthesis, protein structure, and the organelles of the cell.
2. It would be beneficial if students are familiar with the concept of centrifugation and gel electrophoresis.
3. Students should know how to use a micropipette to transfer volume, or an introductory lesson will be required.

Daily Lesson Plans

1. Day 1-3: Protein Trafficking Exploration
2. Day 4: The Who, What, Why, Where, When and How of the Immune System
3. Day 5: Pre-lab Procedure Introduction
4. Day 6-10: Experimental Analysis of MHC Trafficking
5. Day 11: Analysis and Reflection

Formative Assessment

1. Students will create a model of protein trafficking to compare the secretory pathway versus the endosome pathway. The model will be assessment based on the accurate representation of necessary cellular compartments and the effectiveness of the group's presentation of the model.
2. Students should demonstrate their understanding of western blotting and cell fractionation prior to performing the wet lab. This can be done in the format of a quick quiz or exit ticket.
3. Students should demonstrate their understanding of the immune system by creating a flow chart after hearing an introduction using "The Who, What, Why, When, Where, and How of the Immune System" PPT.

Summative Assessment

1. Students will compose a lab report synthesizing the information they learned throughout the unit and analyzing the data that they have collected. The lab report will include a self-generated diagram that displays MHC Class I and Class II trafficking through the cell.

Student's Section

Where did all my proteins go?

An Overview of Protein Trafficking

Each cell in the human body has about 25,000 genes and the potential to create hundreds of thousands of proteins. No cell makes all of these proteins, but it is possible for a single 10 μm cell to have thousands of proteins at any given time. These proteins are able to function because of a specific folding pattern **and** the environment of cellular compartment where they reside.

Part 1: Conduct some research to determine where in the cell the following proteins are localized. Write the protein name in the proper location of the cell diagram below. (NOTE: All of these proteins would not necessarily be expressed in the same cell at the same time)

Major Histocompatibility Complex		Succinate Dehydrogenase	Actin
RNA polymerase	Calreticulin	Tapasin	GM-130

https://www.timvandevall.com/science/animal-cell-diagram/

Part 2: Now let's consider how these proteins get where they need to go…

Oregonstate/edu/instruction/bi314/fall11/proteinsorting.html
Table 15-3

One of the first things to emerge from a ribosome during protein synthesis is known as the **signal sequence**. This signal sequence will be recognized by **chaperone** proteins that help traffic the protein to the proper intracellular location.

From the table above, it is clear that proteins can be temporarily or permanently imported into the ER. Where would a protein likely go after exiting the endoplasmic reticulum? _____

Model the process of protein movement from the endoplasmic reticulum. Use the following description to create your clay model.

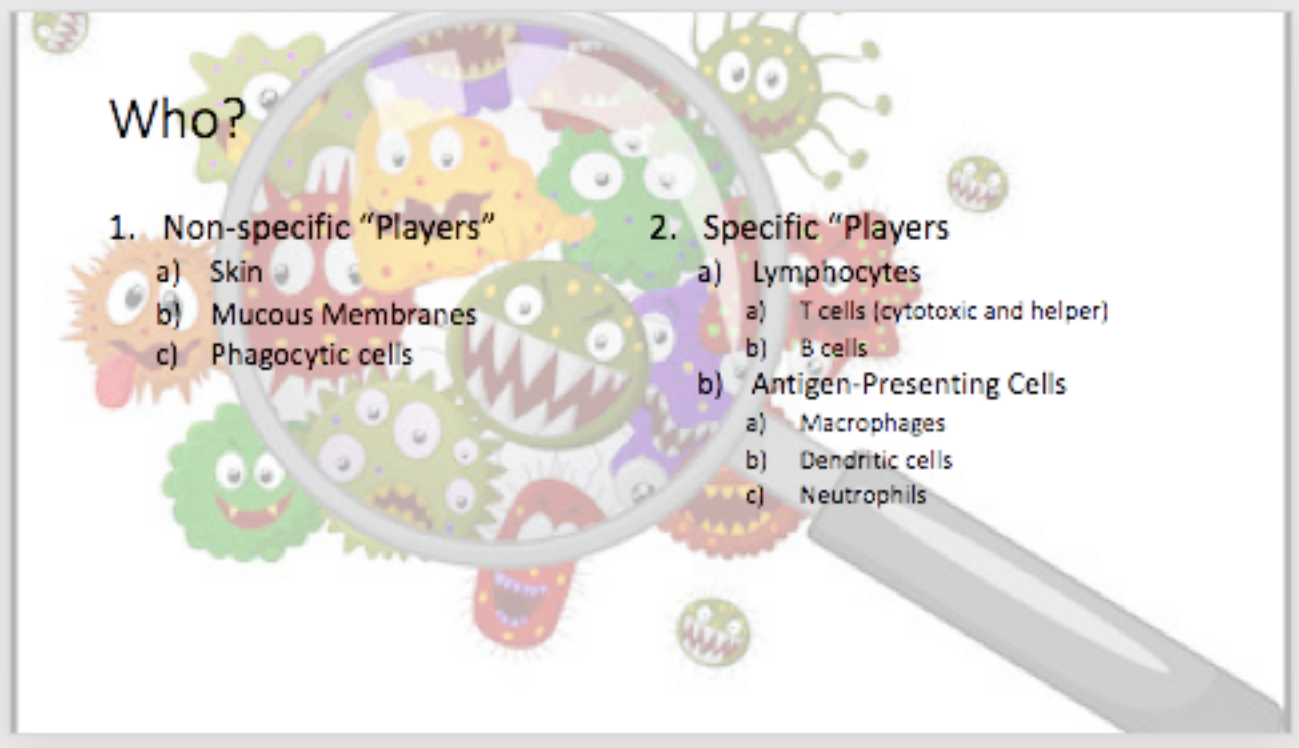
Proteins with an **"Import into ER" signal** are synthesized into the **ER lumen** where they are folded and post-translationally modified. Once these steps are complete the protein is exported in a **vesicle** and sent to the **Golgi apparatus**. The Golgi apparatus is a series of folded membrane compartments that contain enzymes that carry out post-translational modifications such as glycosylation and methylation. Once the protein passes from the **cis membrane to the trans membrane** it is exported from the Golgi in a **vesicle**. This vesicle could move along

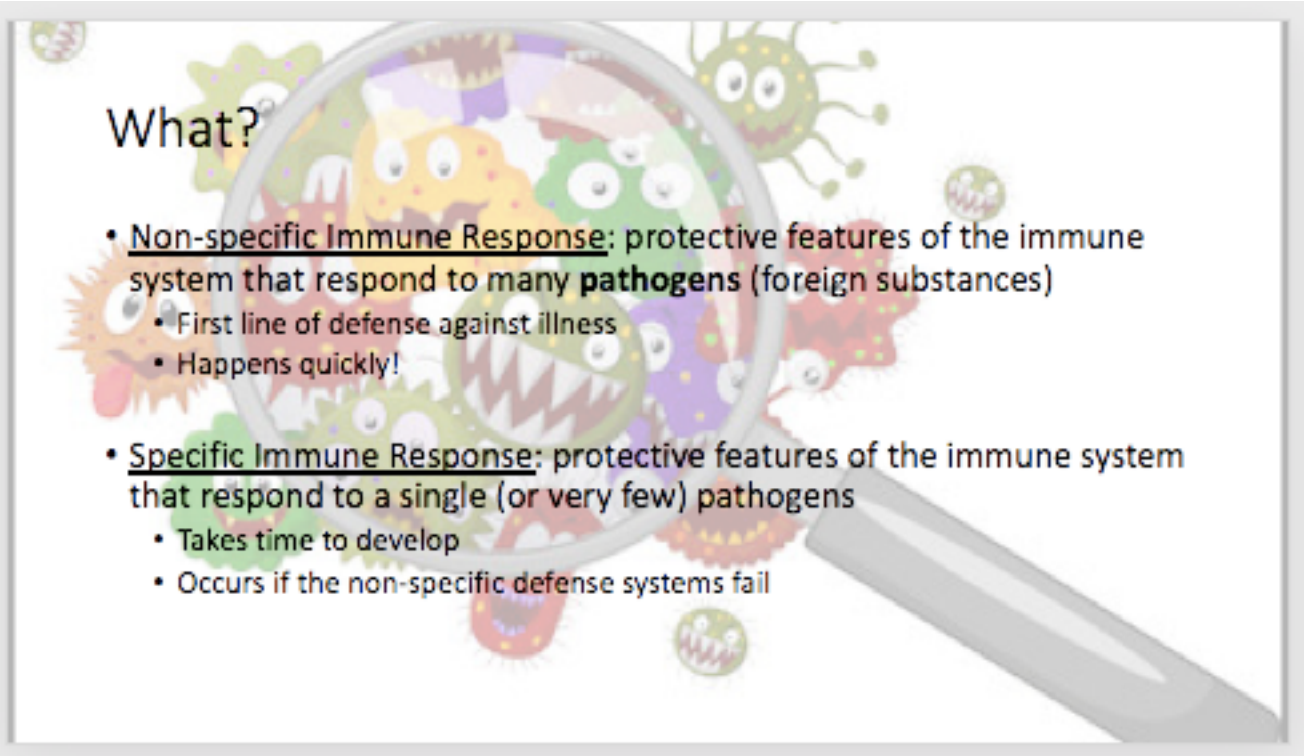
the **cytoskeleton** to the **cell membrane** to export the protein or incorporate the proteins into the membrane. Vesicles that do not go to the Golgi apparatus may also fuse with **endosomes** (which were engulfed by the cell) to **form late endosomes** and **lysosomes**. The proteins that undergo this process are typically involved in molecular hydrolysis.

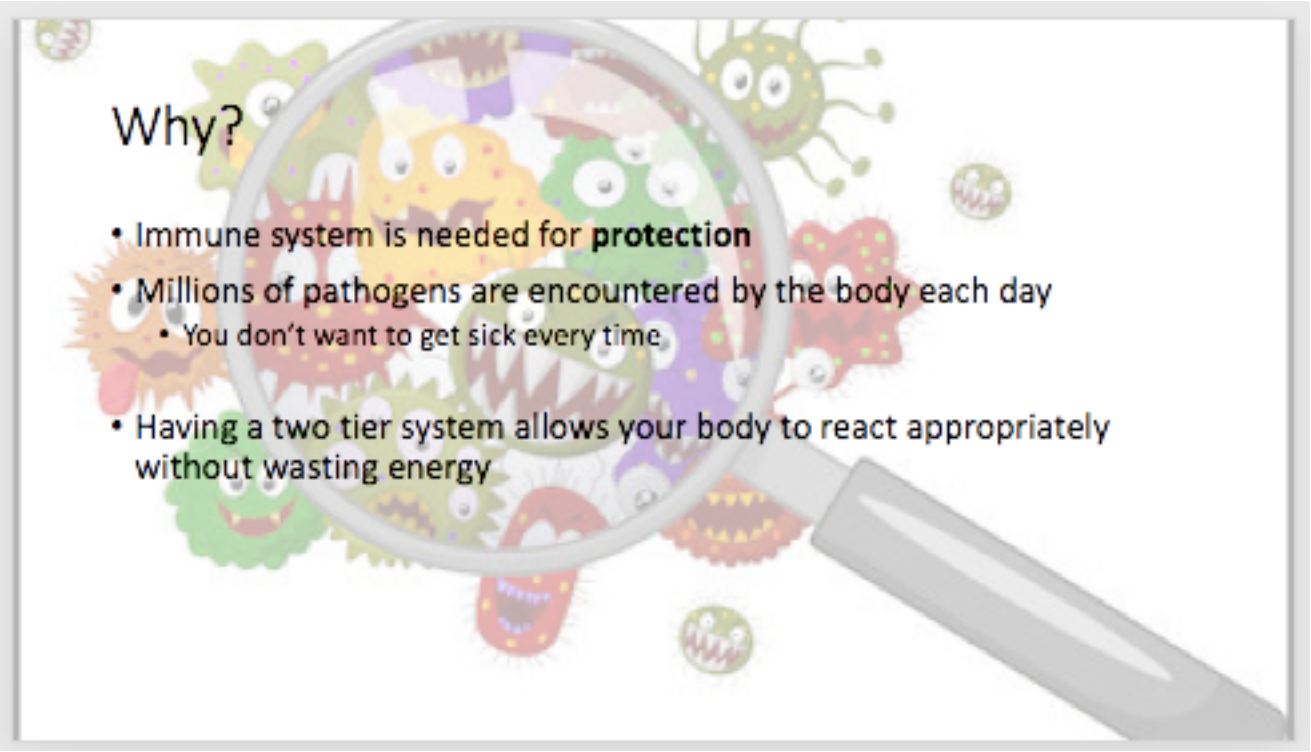
Model Expectations:

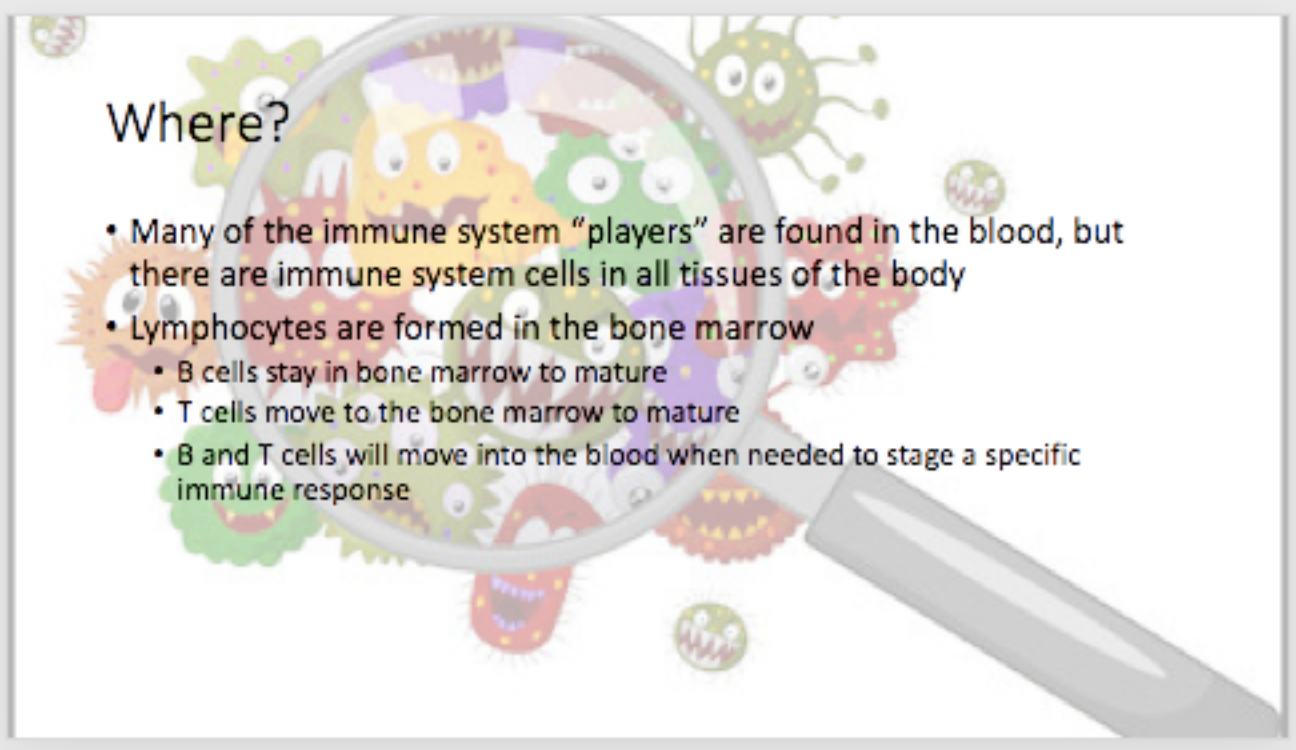
- All bolded terms should be labeled in your model
- You should be able to explain the **progression of steps** without referring to this paper
- All group members should contribute to the explanation

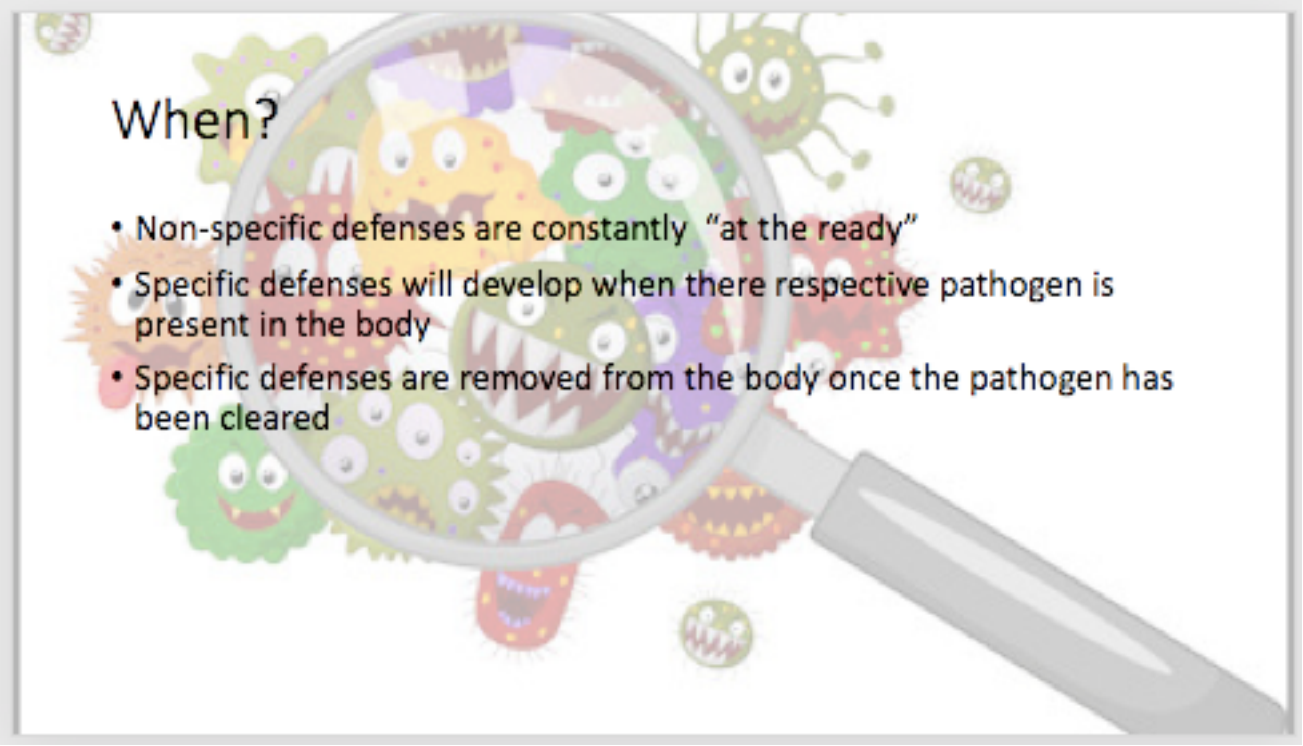

The Who, What, Why, Where, When & How of the Immune System
AAI Unit Plan 2018
J. Katz


Who?
1. Non-specific "Players"
a) Skin
b) Mucous Membranes
c) Phagocytic cells
2. Specific "Players
a) Lymphocytes
a) T cells (cytotoxic and helper)
b) B cells
b) Antigen-Presenting Cells
a) Macrophages
b) Dendritic cells
c) Neutrophils


What?
• Non-specific Immune Response: protective features of the immune system that respond to many **pathogens** (foreign substances)
• First line of defense against illness
• Happens quickly!
• Specific Immune Response: protective features of the immune system that respond to a single (or very few) pathogens
• Takes time to develop
• Occurs if the non-specific defense systems fail


Why?
• Immune system is needed for **protection**
• Millions of pathogens are encountered by the body each day
• You don't want to get sick every time
• Having a two tier system allows your body to react appropriately without wasting energy


Where?
• Many of the immune system "players" are found in the blood, but there are immune system cells in all tissues of the body
• Lymphocytes are formed in the bone marrow
• B cells stay in bone marrow to mature
• T cells move to the bone marrow to mature
• B and T cells will move into the blood when needed to stage a specific immune response


When?
• Non-specific defenses are constantly "at the ready"
• Specific defenses will develop when there respective pathogen is present in the body
• Specific defenses are removed from the body once the pathogen has been cleared

The **major histocompatibility complex** (MHC) proteins play a vital role in the initiation of the **adaptive immune response**. MHC is a cell surface protein found in many cells that present **antigens** to other cells of the immune system. Antigens are small peptide fragments derived from the proteins of invading microorganisms/viruses. When antigens are presented on the cell surface by MHC, immune system cells such as **B and T cells** can recognize them and activate the production of **antibodies** or the **cytotoxic** activity of T cells. Because of the role of MHC in initiating the immune response, it is vital that it is properly trafficked through the cell. It took a great deal of experimentation to determine how the two classes of MHC proteins make it to the cell surface.

The experiments that were conducted involved two key processes, cell fractionation and western blotting. **Cell fractionation** is a process that separates the organelles of a cell based on mass and/or buoyant density. In order to fractionate the organelles in cells, the cells must first be **homogenized**. This requires that the cells are broken open, but the membrane bound compartments are maintained. This is done using a high-speed blender or **sonicator** (sound waves).

Why would you not want to lyse the cell?

Most fractionations begin with differential centrifugation. The image to the below displays this process. **Use the information provided to write a written description of this process.**

https://www.ncbi.nlm.nih.gov/books/NBK21492
Figure 5-23

What physical property is this separation technique based on? _____

Why do you think that differential centrifugation is not the only step required for cell fractionation? Think about the end product of this process.

The second step of cell fractionation typically includes **equilibrium density-gradient centrifugation**.

What physical property do you think this method is based on?_____

In order to carry out this process, the impure organelle fraction from differential centrifugation is placed on top of a gradient of sucrose or glycerol. The tube is then spun at high speeds for several hours until each organelle migrates to equilibrium. **Equilibrium** is reached when the density of the organelle matches the density of the sucrose gradient, as seen in the image to the right.

The endoplasmic reticulum has a density of 1.20 g/cm^3 and the plasma membrane has a density of 1.12 g/cm^3. Include arrows to show where these organelles would appear after centrifugation.

https://www.ncbi.nlm.nih.gov/books/NBK21492/
Figure 5-24

The purity of each fraction can be observed by detecting marker proteins. Proteins in a given extract can be detected using **western blotting**. The process is simplified in the image below.

https://www.novusbio.com/application/western-blotting

Image (A) Proteins are separated on a gel made of poly-acrylamide. Poly-acrylamide forms porous material which molecules can move through when an electric current is applied. The molecules will move based on two properties.

Use the image above, as well as the image below to determine what these two properties are.

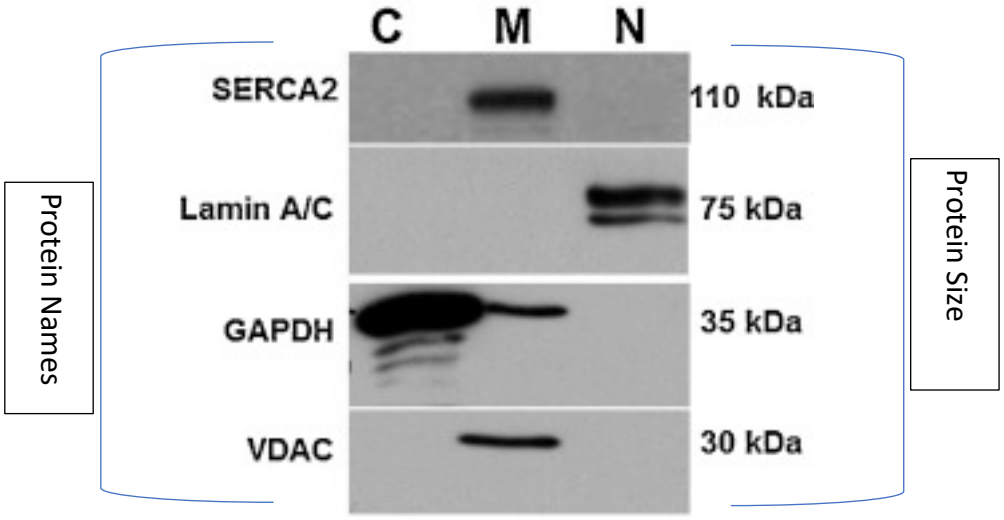
_____ _____

Elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch07s03.html
Figure 7.3

Image (B) The proteins that were separated get "blotted" from the gel onto a membrane, so that they can be detected using **antibodies**. Antibodies are proteins that bind specifically to another protein. In the case of this lab, you will use an antibody specific for a marker protein in four key organelles: Endoplasmic reticulum, endosome, plasma membrane, and Golgi apparatus.

Because proteins are microscopic and cannot be observed with the naked eye, we will not know when the protein has been detected by the antibody, therefore we need to use a secondary antibody that detects the primary antibody. This antibody is bound to horse radish peroxidase, an enzyme that oxidizes several substrates to create a colored product. This color can be detected on the membrane and will tell us that our protein is present.

Use the image below to answer the following questions. C =cytoplasm, M = membrane, N = Nucleus



Which protein included can be used as a marker for the nucleus? _____

What is likely present at the end of Lamin A/C that allowed it to get to the nucleus? _____

Which protein included can be used as a marker for the cytoplasm? _____

Why was VDAC able to move further down the gel than SERCA2?

_____

What is different about the amount of GADPH in the cytoplasm and that in the membrane?

_____

Which protein is absent from the membrane? _____

Explain the likely process taken by GAPDH to get to the membrane.

_____

_____

_____

Experimentally Determine the Intracellular Pathway taken by MHC Class I & Class II

Major Histocompatibility Complex (MHC) proteins are human cell surface proteins vital for the presentation of antigen (protein on the surface of an invader) and initiation of a specific immune response. MHC proteins are divided into two classes based on their structure, MHC Class I and MHC Class II. The two different protein classes are recognized by different immune cells which allows for a slightly different immune response. This is beneficial because certain immune cells are better for fighting bacteria, while others are better for fighting viruses.

It was experimentally determined that MHC Class I and Class II move through cell differently. In this lab activity you will carry out a western blot of cell fractionation extracts to determine the likely pathway followed by each of these proteins.

In order to accurately analyze the gel, we run, you will need to use marker proteins to verify that each extract is enriched for the given organelle. Before starting, conduct research to identify where each of the marker proteins are found within the cell.

Calreticulin: _____

LAMP1: _____

GM-130: _____

Materials:

Total cell extract with SB
ER extract with SB
Endosome extract with SB
Membrane extract with SB
Golgi extract with SB
Anti-Calreticulin
Anti-MHC I
Anti-MHC II
Anti-GM130
Molecular Weight Marker
SDS-PAGE gel (10-20%)
Gel electrophoresis apparatus
Transfer membrane apparatus
Nitrocellulose membrane
200 µl pipette
Pipette tips
SDS-PAGE Running Buffer (10 x)
Methanol
Transfer Buffer
Blocking Buffer
Amplified Opti-4CN Substrate Kit
Goat-Anti-mouse HRP

Procedure:

Part A: Separating Protein

1. Thaw extract (ER, endosome, membrane, Golgi) by placing them in an ice bucket
2. Boil the samples for 5 minutes at 95°C in a hot water bath
3. Spin samples at room temperature for 1 minute at 13,000 RPM

As a class:

4. Fill up gel box half way with SDS-PAGE running buffer
5. Set up 2 SDS-PAGE gel in running apparatus (depends on device and number of wells)
6. Fill the gel box to the fill line with SDS-PAGE running buffer
7. Pull out green well comb
8. Load 10 µl of MW weight marker in Lane 1, 6, 11 and Lane 1 of the second gel
9. Load 20 µl of each extract into the next 4 lanes (Total, ER, Golgi, Membrane)
10. Each group will load their extracts into the gel
11. Run gel for ~1 hour at 200V (watch that the SB does not run off the gel)

Part B: Blotting onto a Nitrocellulose Membrane

While the gel is running:

12. Soak membrane (one per gel) in methanol for 2-3 minutes
13. Soak mesh pads and 6 pieces of filter paper in cold SDS transfer buffer (stored at 4°C)
14. Set up antibody dilutions in blocking solution: (each group will be responsible for one dilution)
    a. Anti-Calreticulin= 1:1000
    b. Anti-GM-130 = 1:400
    c. Anti-MHC I = 1:5,000
    d. Anti-MHC II = 1:5
15. Fill the gel cartridge
    a. Lay down the black side of the cassette
    b. Place in one mesh pad
    c. Lay down 3 pieces of filter paper
    d. Lay down nitrocellulose membrane (**make sure there are no bubbles**)
    e. Remove gel from running apparatus and lay on top of the membrane
    f. Lay down 3 pieces of filter paper
    g. Place in one mesh pad
    h. Close gel cartridge with sliding clamp
    i. Repeat this process for second gel
16. Slide gel cartridge into transfer apparatus with black side towards red electrode
17. Place ice pack in apparatus
18. Fill apparatus with cold transfer buffer
19. Run at 100V for 35 minutes

Part C: Detect proteins

20. Remove membrane from transfer apparatus and place in blocking solution on rocker for 30 minutes
21. Cut the membrane into 5 sections, each containing a MW marker and the four extracts.
22. Incubate each section of membrane with a different antibody overnight at 4°C
23. Pour off antibody and wash each membrane 3x with PBS Tween
24. While washing, dilute the anti-rabbit HRP secondary to 1:2000 in blocking solution
25. Incubate each membrane at room temperature for 1 hour in secondary antibody
26. Pour off antibody
27. Wash three times with PBS Tween

Part D: Develop Image

28. Dilute the Amplified Opti-4CN Substrate according to directions in kit
29. Develop membranes in Amplified Opti-4CN Substrate for 30 minutes on rocker
30. Reassemble membranes and take picture

Analysis (Please type following):

You will compose a laboratory report to synthesize the information gained from both the introductory activities and the experimental analysis. Your lab report will include the sections listed below. Remember that lab reports require outside research to expand upon your results and suggest future direction.

1. Introduction:
    a. Discuss the organization of the immune system
    b. Introduce the role that protein trafficking plays in its accurate functioning
    c. Introduce the methods that were utilized
2. Methodology
    a. Make sure that this is IN YOUR OWN WORDS
    b. Explain it so a professional in the field could repeat your procedure
3. Results:
    a. Present labeled gel images with appropriate titles and captions
    b. Provide a written description of the results
4. Discussion
    a. Explain what the results on the membrane show you
        i. Be sure to discuss the need for each marker protein
        ii. Do not ignore any results! If you see things that you did not expect be sure to explain why that could have occurred.
    b. Provide a diagram that outlines the difference between MHC class I and MHC class II movement in the cellar (Make sure that is it properly labeled)
    c. Conduct research to determine why the proper trafficking of these proteins in important—think about looking into diseases associated with mis-trafficking.

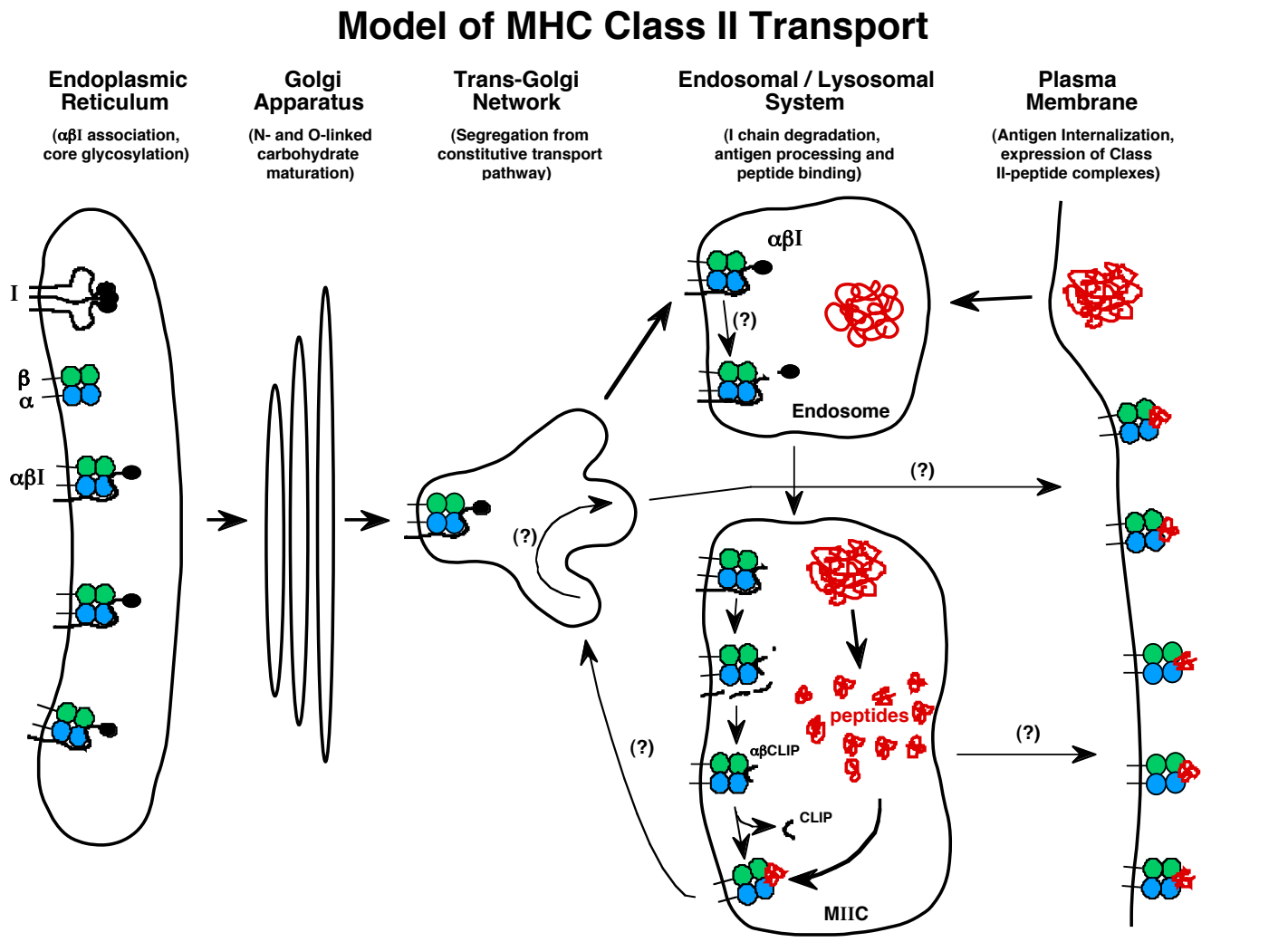
Lab Report Rubric

Category	Total Points	Points Earned & Comments
Introduction • Start with the purpose of the lab • Provides background needed to understand results (see above) • Clear and concise; one topic flows into the next	15	
Procedure • Written in paragraph form (NO STEPS) • Describes all procedures performed • Bolded materials • Contains sufficient detail to allow a reader **who works in the field** to understand what you did to collect data	10	
Results • Written description of results • Properly labeled membrane image	15	
Discussion • Refers to specific data points/figures (incorporates the images that outline MHC movement) • Includes interpretation of results (see above) • Includes explanations of unexpected results (if applicable) • Includes potential next steps • Summary statement • One topic flows to the next	20	
Works Cited • Includes all sources consulted • Aligned with in text citations	5	
Formatting & Conventions • Each section is labeled • Written in past tense • Few grammar and spelling mistakes • Document is properly titled when uploaded to Google Drive	5	

Total Points: _____/70

The Trafficking of Major Histocompatibility Protein Class II (Extension Activity)

Like other proteins, MHC Class II is trafficked through the ER and Golgi apparatus before it docks on the cell membrane. The diagram below shows the process taken by MHC Class II (shown as green and blue).



Lisa K. Denzin

Use the diagram above to answer the following questions.

1. Based on your observations how many subunits is the MHC Class II protein made of? _____
2. In which compartment is MHC Class II assembled and partially modified?
   _____
3. Which other protein seems to associate with MHC Class II in the endoplasmic reticulum?
   _____
   a. What are some potential functions of this second protein?

_____

_____

4. What might be present in the cytoplasmic "tail" of this protein?

_____

_____

_____

5. What does the red "blob" in the endosome represent? _____

6. How did this red "blob" enter the cell? _____

7. When an endosome and a Golgi vesicle containing MHC Class II merge they are referred to as MIIC (MHC Class II Compartment). Explain what is happening in this compartment.

_____

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_____

8. Where does MHC Class II go after the MIIC? _____

9. What will happen in the body if MHC Class II is presenting a bacterial/viral peptide?

_____

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10. Make a prediction: What do you think would happen to an organism if the MHC Class II had a mutated ER import signal sequence. Think about the effects in **terms of both protein trafficking and the organism's immune system**.

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