Name:

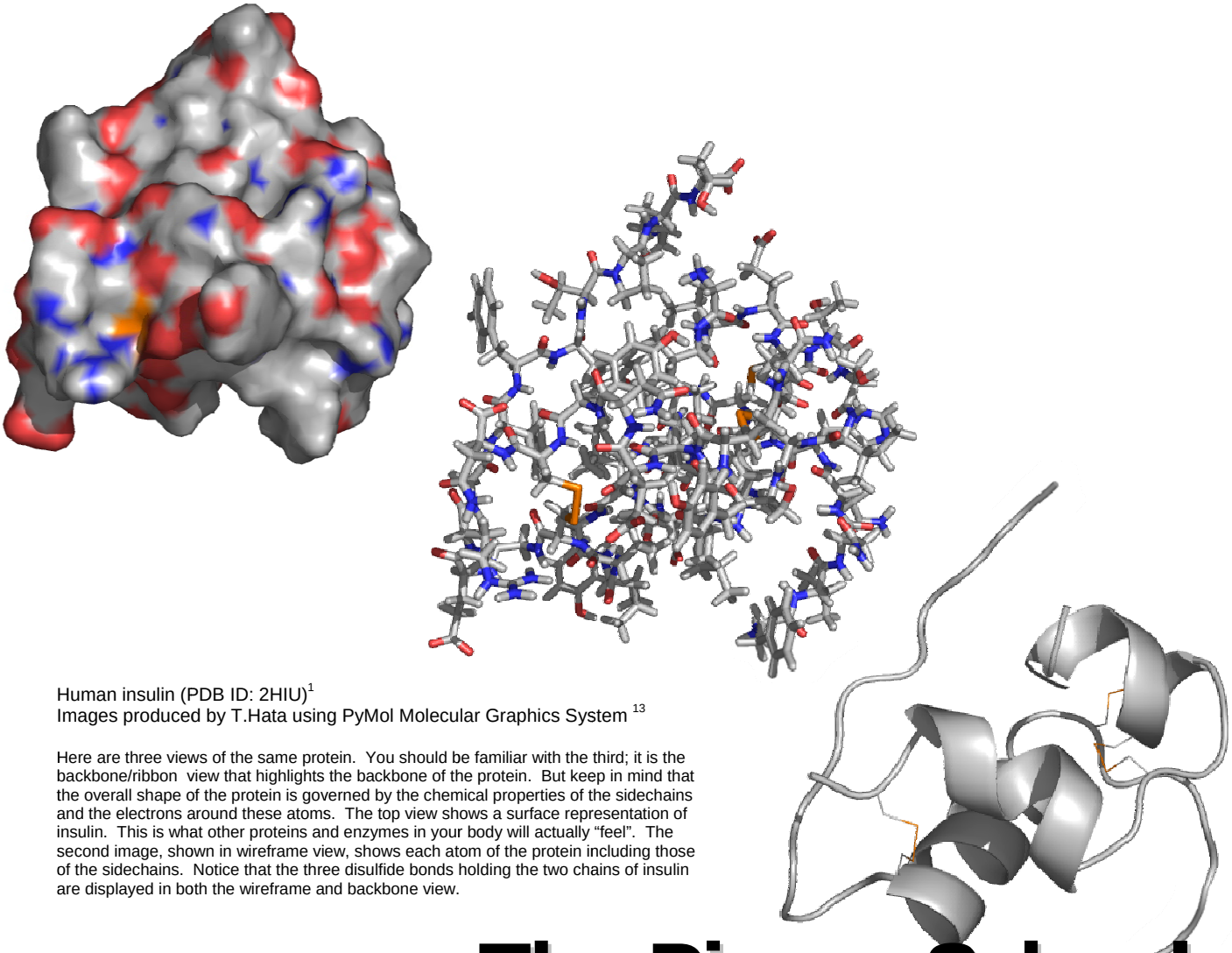
Distributed January 11, 2007
Quiz available Sunday, January 27, 2007
Quiz must be completed by Wednesday, January 30, 2007

Project

2

PROTEINS AND CELLULAR FUNCTIONS

Transport across a membrane & cellular secretion of a protein



Human insulin (PDB ID: 2HIU)[1]
Images produced by T.Hata using PyMol Molecular Graphics System [13]

Here are three views of the same protein. You should be familiar with the third; it is the backbone/ribbon view that highlights the backbone of the protein. But keep in mind that the overall shape of the protein is governed by the chemical properties of the sidechains and the electrons around these atoms. The top view shows a surface representation of insulin. This is what other proteins and enzymes in your body will actually "feel". The second image, shown in wireframe view, shows each atom of the protein including those of the sidechains. Notice that the three disulfide bonds holding the two chains of insulin are displayed in both the wireframe and backbone view.

The Pingry School Biology Honors Projects

http://www.pingrybiology.com/honors_projects.htm

Introduction

Reflection of Project 1

Through Project 1, you were introduced to a number of tools used by scientists to study and view protein structure. We hope that the modules and the use of the PDB website and Jmol has supplemented your understanding of how protein structure is related to function.

You should be familiar with the basic concepts of protein structure. You saw that serum albumin, with its abundant disulfide bonds, allows it to remain stable in the harsh and unstable chemical environment of the blood. You also saw how the chemical properties of the sidechains lining the binding sites of were responsible for the protein's ability to bind nonpolar molecules such as fatty acids.

You should also be able to access and understand the structural characteristics of the structures deposited in the PDB.

Project 2 Overview

In class, you have been discussing the structures and functions of a cell. You should be familiar with the mechanisms through which select molecules are transported across the membranes of the cell. In this module, you will explore the structure of two channel proteins that permit the movement of ions and water molecules across the cell membrane.

These transmembrane proteins are created by the ribosome, processes and packaged by the rough ER and golgi apparatus, and ultimately placed in the cell membrane as a transport vesicle from the golgi apparatus fuses with the cell membrane. In contrast, other proteins also produced by the ribosome and processed in the ER and golgi apparatus are destined for other organelles in the cell or are secreted out of the cell. What determines where each protein ends up?

You were told in class that some of the functions of the ER and golgi apparatus are to "process" the protein made by the ribosome. What does "processing" mean? What are some of the ways that a protein is "processed"?

To explore the pathways of protein processing and secretion, you will look at how insulin is produced. You will also revisit the PDB resources to build a model of insulin.

Submission Instructions

Students are expected to work independently on all parts of the project except for portions that are specifically noted to be completed with a partner.

You must read the packet carefully and completely. Follow any modules that instruct you to examine resources on the web and instructions on how to navigate these web resources. At the end of the first part of this project, you will take an online quiz. Follow directions when completing the quiz! Failure to follow instructions may result in the rejection of your quiz; leading to a mark of "incomplete" for Project 2.

During the initial posting of the quiz, you are allowed one (1) attempt at the quiz. Unlike the quiz you took for Project 1, you will no longer immediately receive your score when you submit the quiz. Again, you are to take the quiz just once. Taking the quiz repeatedly is a violation of the instructions. Also keep in mind that the quiz is to be completed individually. You may **not** consult with other students or other unauthorized resources.

At the beginning of Part 2, you will be paired with a partner from another class to complete the insulin model building project.

Outline of Project 2

Module 2.1

A closer look at transport through the membrane - the potassium (K^+) channel and aquaporin

Module 2.2

Cellular protein targeting

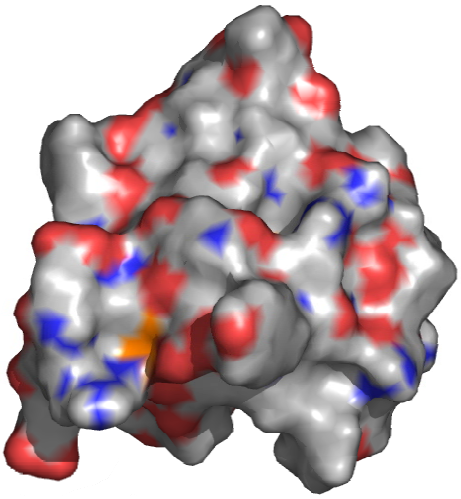
Module 2.3

Protein sorting, modification, and secretion: insulin

Online Quiz

Two attempts

Passing grade 8/10



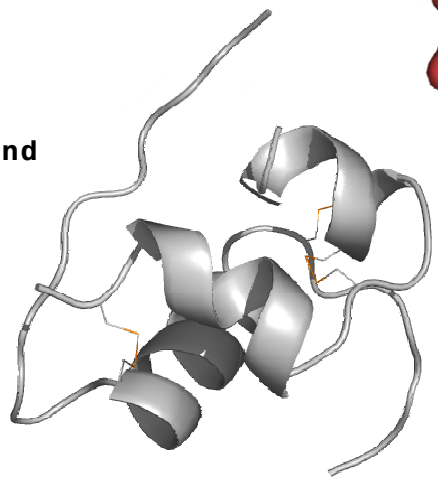


Fig 1: Human insulin (PDB ID: 1HIU). The image of insulin to the left is shown highlighting only the protein backbone and ribbon view used to highlight the alpha-helices. Although you are familiar with this view, no not forget that the overall shape of a protein is also determined by the structure of the sidechains and the electrons around each atom. The image above shows the chemical "surface" of insulin in the same orientation as the image to the left. This better represents what other proteins and enzyme actually "feel" when interacting with insulin. As you continue to encounter images of proteins and enzymes, make sure you don't lose sight of the advantages and disadvantages of each view.

Module 2.1

A closer look at transport through the membrane - the potassium (K^+) channel and aquaporin

Cells are the fundamental unit of all organisms. From the simplest prokaryotic cell to a cell of a multi-cellular eukaryote, all cells share a number of common characteristics. One of these characteristics is the cell membrane composed of two layers of phospholipids referred to as the phospholipid bilayer.

The cell membrane separates the intracellular environment from the extracellular environment. The ability of this membrane to limit and control the movement of molecules in and out of the cell is crucial for life. Recall from your class discussion that one type of transmembrane proteins function as "channels" to allow the selective passage of molecules across the cell membrane. As you might already guess, the basis of this selectivity of these channels is their individual structures. You may have played with toys that operate in a similar way; the Shape-O Toy© by Tupperware®.

Figure 2: Shape-O Toy© demonstrates how specific shapes of the openings (structure) determine which piece can fit through (function). Similarly, the structure of membrane channels determine what molecules are able to pass through the membrane.[9]

Exactly how certain membrane proteins are capable of selectively moving molecules across the membrane was not understood until fairly recent discoveries. Two of these discoveries came from work done by two scientists' labs: Dr. Peter Agre from Johns Hopkins University and Dr. Roderick MacKinnon from Rockefeller University. The two scientists were awarded the 2003 Nobel Prize in Chemistry for discovery and identification of the water channel (aquaporin) in 1990 and structural studies of ion channels in 1998, respectively. Since their discovery, scientists have also correlated the cause of some diseases to errors and malfunctions of channel proteins. Research surrounding these channel proteins continues to change our understanding of how the cell works.

Let's think a moment about the Shape-O Toy© shown above. One of the holes on the toy sphere have the shape of a circle. Because of this shape, only the "circle piece" can be fit

through this hole. If you were playing with this toy, you could not fit any other piece through. But imagine that you had a bag of marbles; you could easily fit marbles through any of the larger openings on the toy.

You would think that channel proteins face a similar challenge. Two common ions that pass through the membrane are the Potassium (K^+) and Sodium (Na^+) ions. Controlling movement of these ions through the cell membrane is related to many biological functions including the sending of signals through nerve cells through your body. Accordingly, there are a number of channels and pumps that facilitate this movement. One of these channels is known as the Potassium Channel. This transmembrane protein is responsible for the movement of K^+ ions into the cell. But surprisingly, it does not allow Na^+ ions to pass through. Why is this surprising? Na^+ ions are smaller than K^+ ions, like marbles compared to the larger "circle piece."

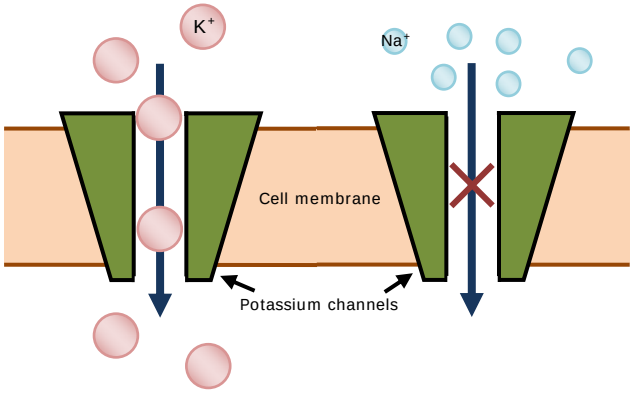


Figure 3: The potassium channel allows the larger potassium ion to go through but prevents the smaller sodium ion from go through. The structural basis of specificity must be more than simply the size of the pore.

How is this selectivity for ions possible?

Access the Nobel *e*-Museum at http://www.nobel.se/index.html. Navigate to the "Nobel Prizes" section using the top menu bar (far left) and continue to the "Nobel Prize in Chemistry" link that shows up immediately below the main menu. Then look for the "All Nobel Laureates in Chemistry" link that is in the column towards the right of the page below the heading "The Nobel Prize in Chemistry". You should see Dr. Agre and Dr. MacKinnon listed under "2003." Access their page. (website layout current as of January 2008)

On this page, there are two modules you need to read. Both are listed in the column to the right side of the page; "Press Release" and "Information for the Public." These two pages provide a general explanation of the biochemical mechanism of the two channel proteins.

Read these two modules.

After reading the modules on the Nobel *e*-Museum website, go to the PDB website to access the "Molecules of the Month" feature on the Potassium Channel. You should remember how to access this resource from Project 1. This "Molecule of the Month" module will further discuss the structural significance of the channel and will supplement your reading from the Nobel *e*-Museum website.

What are some of the structural characteristics of the Potassium Channel that explains its selectivity for potassium ions? How are the protein's sidechains involved in this process? What happens when the channel is inhibited?

For our discussion of the Aquaporin, it may be appropriate to point out an old debate that has been going on within the science education community regarding the movement of water across the cell membrane. Textbooks from just a few years ago state that water is able to simply "diffuse" through the plasma membrane. It argues that although polar, water is small enough to squeeze through the hydrophobic tails of the lipid bilayer. Since the identification of the water channel (aquaporin), some textbooks have now introduced the fact that water can ALSO move through channels; thus ALSO go through passive facilitated diffusion. Yet, there is little written to revisit the earlier notion that water is able to freely move through the lipid bilayer. There is still some conversation arguing whether or not water freely diffuses through the cell membrane. A simple search on Google with the phrase "can water diffuse through the cell membrane" will lead you to a number of biology

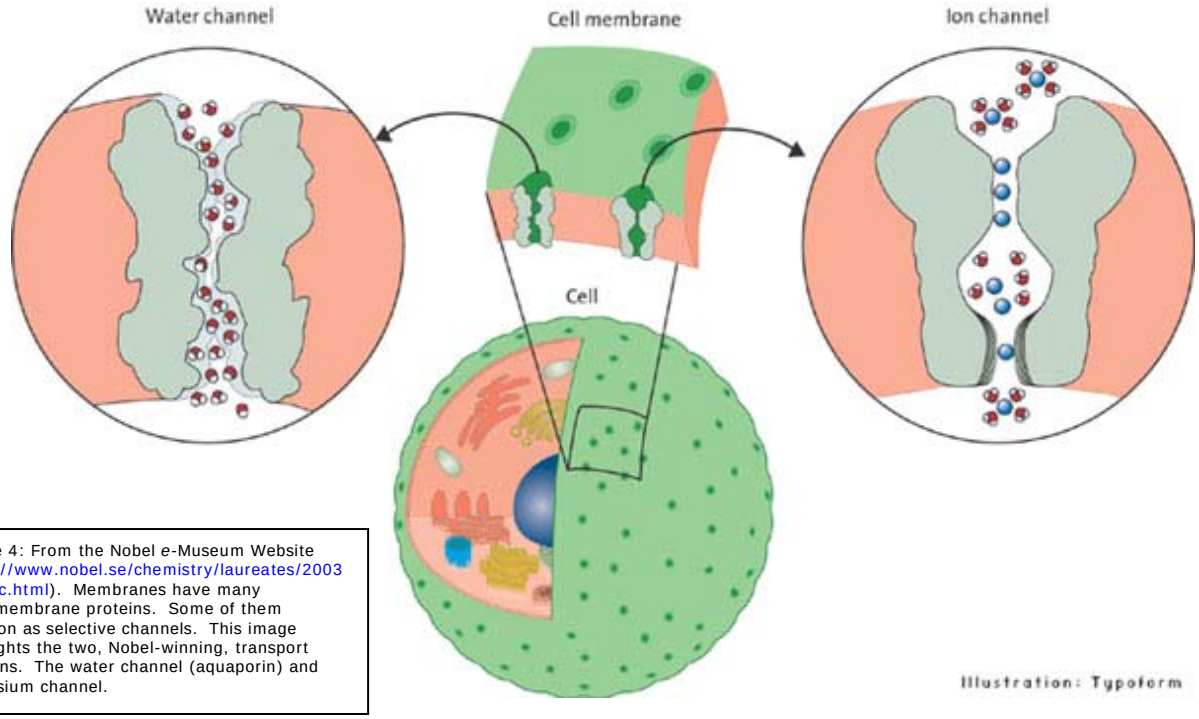


Figure 4: From the Nobel *e*-Museum Website (http://www.nobel.se/chemistry/laureates/2003/public.html). Membranes have many transmembrane proteins. Some of them function as selective channels. This image highlights the two, Nobel-winning, transport proteins. The water channel (aquaporin) and potassium channel.

related websites and archived lecture outlines mentioning that “water does freely move through the membrane.”

As you read in the Nobel *e*-Museum module, one of the results from Dr. Agre’s experiments point out a definite answer to the question. This is a great example of how science could “change” our understanding of biological systems and how things in our world work. Considering that popular biology textbooks, such as the one you use in class, are updated every two to three years, such significant advances in our understanding of biological processes seem to take a while to trickle down into textbooks (remember that Dr. Agre published his discovery well before being awarded the Nobel Prize). Meanwhile, there are many exciting advances in the scientific field that continue to change the way we look at how things work in our cells and bodies.

How does Dr. Agre’s research, discussed on the Nobel website, provide strong evidence that water is only capable of passing through the cell membrane in the presence of aquaporins? Do all cells have the same amount of aquaporins in their membrane? How can the type and amount of aquaporins in the cell membrane affect the properties and functions of the cell?

Module 2.2

Cellular protein targeting

During your class discussion of the functions of the endoplasmic reticulum and the golgi apparatus, you were told that they play a role in "protein modification and sorting". What does this mean? Why is this important? How is this accomplished? We will explore these topics through the next two modules.

A typical mammalian cell can contain more than 10,000 different kinds of proteins. For a cell to function properly, each of its numerous proteins must be transported and placed in the correct cellular membrane or intracellular compartment; the mitochondrial matrix, chloroplast stroma, lysosomal lumen (inside the lysosome), or free flowing in the cytosol (the liquid portion of the cytoplasm). Hormone receptor proteins, for example, must be delivered to the plasma membrane if the cell is to recognize hormones and specific ion-channels and transporter proteins are needed if the cell is to import or export the corresponding small ions and small molecules. Enzymes such as RNA and DNA polymerases (responsible for processes involving DNA) must be targeted to the nucleus; still others, such as proteolytic enzymes or catalase, must go to lysosomes or peroxisomes, respectively. Many proteins, such as hormones and other extracellular proteins you have already explored, including serum albumin, must be directed to the cell surface through transport vesicles and secreted.

How does the cell make sure that all of the proteins end up in the right place?

The process of directing each newly made polypeptide to a particular destination, referred to as protein targeting or protein sorting, is critical to the organization and functioning of eukaryotic cells. This process occurs at several levels and will be described in the next few paragraphs. Recall that ribosomes, the machinery that produces polypeptides, can be found in one of three general locations: (1) on the surface of the rough ER, with newly formed polypeptide entering the lumen of the ER; (2) free flowing in the cytoplasm, with newly formed polypeptides also becoming free flowing in the cytoplasm; and (3) within the two DNA containing organelles, the mitochondria and chloroplast (we won't worry about the third in this project).

Proteins in the organelles, transmembrane proteins in the membranes, and proteins secreted out of eukaryotic cells are all encoded by DNA in the nucleus and synthesized on ribosomes in the cytoplasm (there are a few exceptions). For our purpose here, keep in mind that DNA stores the information to make proteins. These instructions are copied and carried out of the nucleus on a molecule of RNA that is "decoded" by the ribosome to make proteins. Once the ribosomes create these proteins, the proteins are distributed to their correct destinations through the sequential action of sorting signals and multiple sorting events that occur through the endoplasmic reticulum and golgi apparatus.

Before continuing, let's clarify a characteristic of "unprocessed" proteins in the cell that is being created by the ribosome. Not all of the amino acids in the peptide chains being synthesized by the ribosome is part of the actual, functional protein. Some parts, or some peptide regions, have specific roles including signals that determine where the protein will end up. In other words, there are sequences of amino acids attached to the rest of the "protein" that tells the cell to "put in the cell membrane", "transport to the mitochondria", or "secrete out of the cell".

At an earlier stage of protein production, there must even be a signal that determines whether the protein will be created while the ribosome remains free floating in the cytosol or if it will continue on the endoplasmic reticulum. These amino acid sequences are called *signal peptides*.

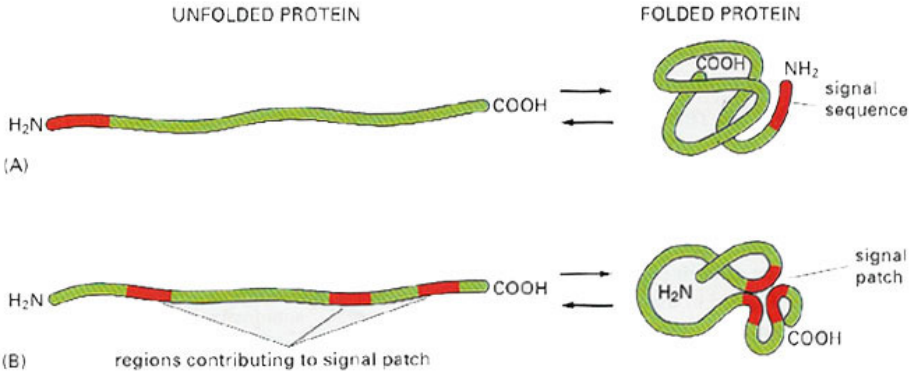


Figure 5: Two ways in which a signal peptide can be built into a protein:
(A) The signal residues in a single stretch of amino acids, called a signal sequence that is exposed in a folded protein.
(B) A signal patch can be formed by the combining of amino acids from regions that are physically separated in the primary structure before the protein folds.
Diagram copied for educational use and distribution [7]

Function of Signal Peptide Sequence	Example of Signal Peptide Sequence
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-COO^-
Import into mitochondria	^+H_3N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into peroxisomes	-Ser-Lys-Leu-COO^-
Import into ER	^+H_3N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln

Review the diagram on the next page step by step as you read through the following paragraphs. In order to understand the diagram, you will probably need to read and review the text on this page and the diagram a few times.

All protein production begins on the cytosolic ribosomes (free flowing ribosomes in the cytosol) as the ribosomes begin synthesizing a short peptide chain. Some of these initial peptide sequences contain a specific signal sequence that directs the ribosomes to the surface of the endoplasmic reticulum (ER). Protein synthesis is then completed as the ribosomes are attached to the ER membrane and the resulting protein is produced into ER. From the ER, the completed proteins move to the golgi apparatus and subsequently are sorted to various destinations. Proteins synthesized and sorted in this pathway include those that are secreted from the cell, the enzymes and other "resident" proteins in the lumen of the ER, Golgi, nucleus, and lysosomes as well as transmembrane proteins in the membranes of these organelles and the cell membrane.

Synthesis of other proteins is completed on "free" cytosolic ribosomes, and the completed proteins are released into the cytosol. These proteins remain in the cytosol unless they contain a specific signal sequence that directs them to different organelles. Many of these proteins are subsequently sorted further to reach their correct destinations within these organelles; these secondary sorting events depend on additional signal sequences within the protein. Each sorting event involves binding of a signal peptide to one or more receptor proteins on the surface or interior of the organelle[11]. Figures on the next page are available for download in color from http://www.pingrybiology.com/honors_projects.htm. You should view them in color for better understanding of the following explanation.

There are also two video clips that supplement this section; view them before moving on through the project.

Keep in mind that each signal peptide has unique chemical and structural properties. Specific receptor proteins are thus able to identify and interact their respective proteins (the signals bind to the receptors). In essence, the signal peptide acts as the ZIP code for each protein "package".

Are you able to describe the pathway of protein production and the role of signal peptides for this process?

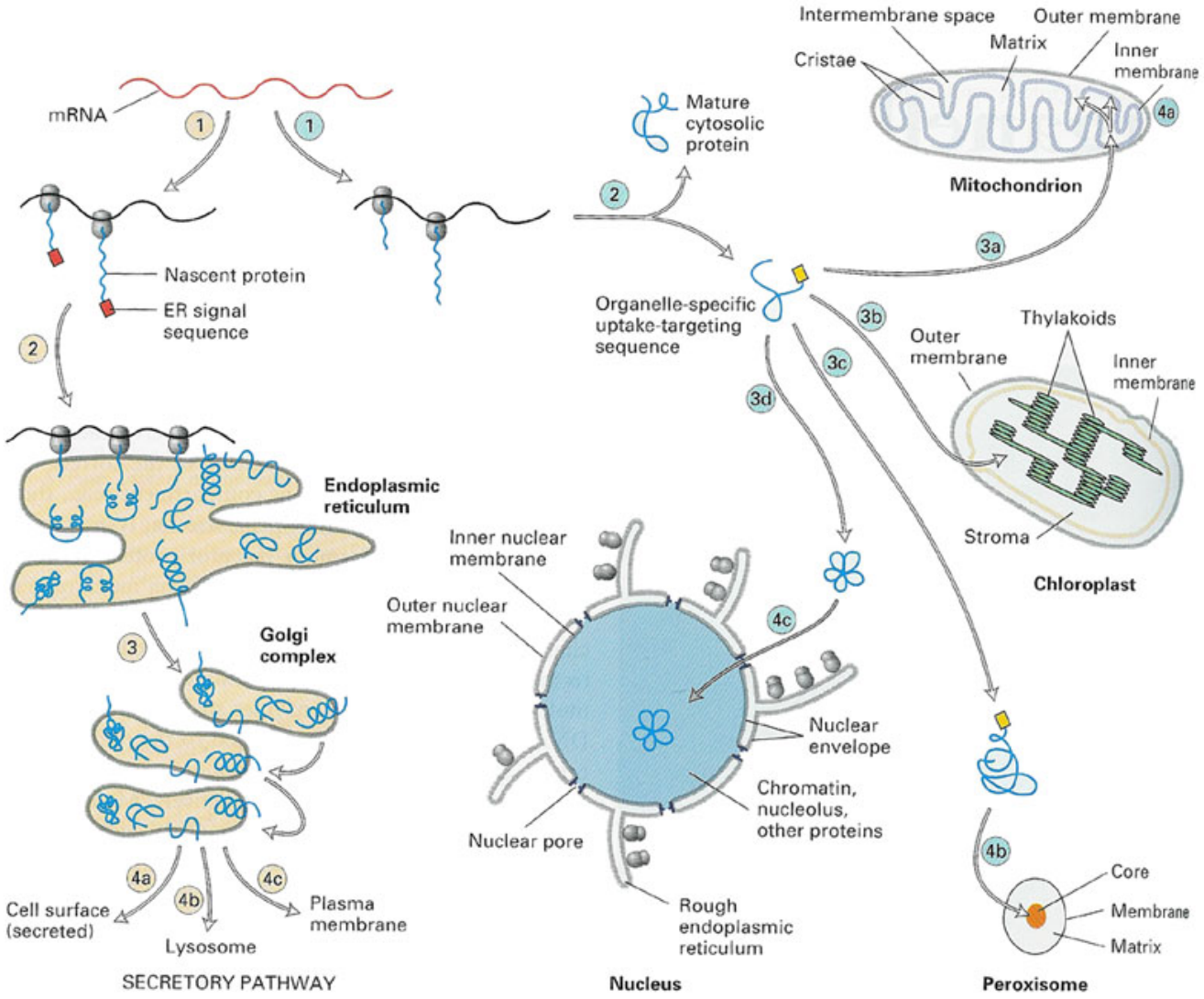


Figure 6: Overview of sorting of nuclear-encoded proteins in eukaryotic cells.
Alberts, B., A. Johnson, et al. Molecular Biology of the Cell: Fourth Ed.. Garland Science, 2002.
Diagram copied for educational use and distribution [7]

mRNA is a nucleic acid responsible for copying the protein building "instructions" from DNA and carrying it out into the cytoplasm. Ribosomes use these instructions to build polypeptides with specific sequences. Ribosomes synthesizing proteins in the secretory pathway (shown in the left and numbers colored yellow) are directed to the rough ER by an ER signal peptide (2). After the protein is synthesized in the ER, these proteins move via transport vesicles to the Golgi complex (3) from whence they are further sorted to several destinations (4).

After synthesis of proteins lacking an ER signal peptide (1) (shown in the right and numbers colored blue) is completed on free ribosomes, the proteins are released into the cytosol (2). Those with an organelle-specific uptake-targeting signal peptide sequence are imported into the appropriate organelle (3). Mitochondrial and chloroplast proteins typically pass through the outer and inner membranes to enter the matrix of the stromal space, respectively. Some remain there, and some (4a) are sorted to other organellar compartments. Unlike mitochondrial and chloroplast proteins, which are imported in a partially unfolded form, most peroxisomal proteins cross the peroxisome membrane as fully folded proteins (4b). Folded nuclear proteins enter through nuclear pores (4c).

Lodish, H., A. Berk, et al. Molecular Cell Biology: 4th Ed.. W.H. Freeman and Co. 2000.

Module 2.3

Protein sorting, modification, and secretion: Insulin

Insulin in a peptide hormone (it is a protein-based hormone, some hormones are lipid-based) that is released by the pancreas. In other words, specific cells in the pancreas must be producing and secreting insulin in the same process that you explored in the last section of this Project.

How is insulin "processed" in the cell to be active and signaled to be secreted out of the cell?

You should already be reminding yourself that insulin is secreted out because of a signal peptide that instructs the ribosome to attach to the ER surface, putting insulin into the secretion pathway. Here, we're review some online resources to get a better idea of how insulin is processed before being secreted.

Read the following two tutorials about insulin.

1. The PDB "Molecule of the Month" feature on Insulin
    - Look up PDB structure 2HIU (Human Insulin). **How many amino acids make up this active, mature insulin? How many chains make up insulin? How are these chains held together?**
2. The "insulin" entry on Wikipedia (www.wikipedia.com). You can simply type in the address, http://en.wikipedia.org/wiki/Insulin.
    - Read the following sections: "Introduction", "Discovery and characterization", and "Structure and production". Pay careful attention to the diagram located next to the "Structure and production" section. The image to the right can be viewed at higher resolution on the Wikipedia website.

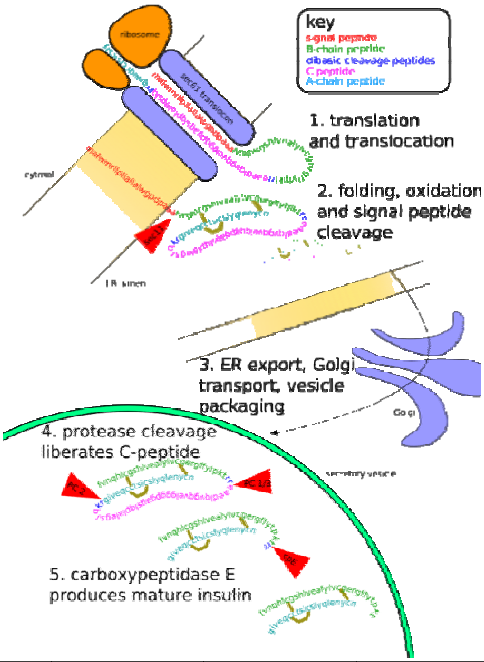


Figure 6: Insulin production and secretion. Image created by Isaac Yonemoto. Wikipedia. Notice the signal peptide highlighted in this diagram. Compare this diagram to Figure 5.

As seen in the diagram in the Wikipedia entry, insulin is first synthesized by the ribosome as proinsulin, a longer protein that includes the C-chain. Also notice the signal peptide region that is responsible for causing insulin to go through the secretory pathway. Mature insulin results from the B-chain and A-chain.

Proinsulin as produced by the ribosome

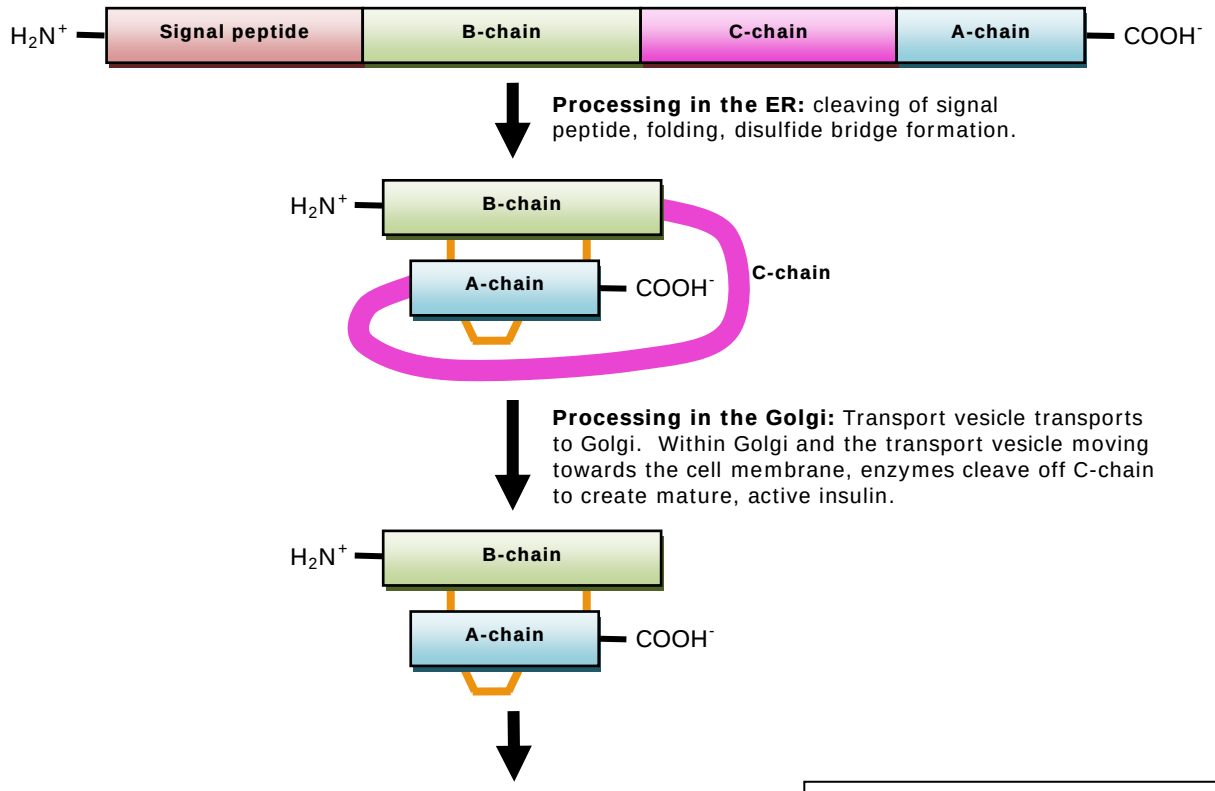


Figure 7: Insulin production and secretion.
A simplified diagram of insulin "modification" before secretion out of a pancreatic cell.

We describe in class that the ER and Golgi are involved in protein "processing". Be able to explain and give examples of how a protein can be "processed" before secretion from the cell. How is the signal peptide involved in insulin secetion?

Online quiz:
Successful students usually report that they spent significant amounts of time carefully reviewing the project before attempting the online quiz. You should have been reviewing this packet well in advance of the availability of the quiz and addressed any questions with your teacher. Good luck.

Notes and Bibliography

1. PDB ID: 1HIU
   Hua, Q.X., S.N. Gozani, R.E. Chance, J.A. Hoffmann, B.H. Frank, and M.A. Weiss. Structure of a protein in a kinetic trap. *Nat.Struct.Biol*. **2** 129-138 (1995)

2. Research Collaboratory for Structural Bioinformatics. Berman H.M. , J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, and P.E. Bourne. The Protein Data Bank. *Nucleic Acids Research*, **28** 235-242 (2000).

3. "Molecule of the Month" features are illustrated and written by David S. Goodsell of the Scripps Research Institute and Shuchismita Dutta at the Protein Data Bank.

4. Shape-O Toy© is a registered trademark of Tupperware®

5. The Nobel *e*-Museum. http://www.nobel.se. 2003.

6. Lodish, H., A. Berk, et al. Molecular Cell Biology: 4th Ed.. W.H. Freeman and Co. 2000.

7. Alberts, B., A. Johnson, et al. Molecular Biology of the Cell: Fourth Ed.. Garland Science, 2002.

8. DeLano, Warren L., "The PyMOL Molecular Graphics System." DeLano Scientific LLC, San Carols, CA, USA. http://www.pymol.org

The Pingry School Biology Honors Projects were developed and written by Tommie S. Hata during the 2003-2004 school year and edited each year by the biology teachers. The Projects are being rewritten in 2006-2007 to reflect current findings in biology and to better reflect topics that we believe is important for our students. The Honors Projects will not be possible without the help from and dedication of the other Pingry biology teachers who continue to offer ideas and suggestions. A special thank you to Deirdre O'Mara for all the input and editing. Thank you also to Dr. Tim Herman, Dr. Mike Patrick, and others at the Center for Biomolecular Modeling at MSOE and the many other scientists that continue to provide us with the technical and intellectual support to make the Projects possible.