Biology 200
Unit 5 Problem Set
The Endomembrane System

Part 1. Test Your Understanding

The following questions focus on review of course content, to help you think critically about what you've learned. Some of these are easier and some are more challenging, but most of them can be answered by using the course material (lecture and textbook). A question's difficulty rating is noted by the 1 (easiest), 2 or 3 (hardest) in brackets beside the question. You should expect to look these up as you go.

Topic 5.1 & 5.2 – The Endomembrane System and Protein Targeting to the ER

a) Define the following terms and determine which ones might be synonyms of each other (1):
   - Signal sequence
   - N-terminal signal sequence
   - Signal recognition particle
   - Signal recognition particle receptor
   - Signal peptidase
   - Start transfer sequence
   - Stop transfer sequence

b) Explain the idea of a cellular compartment, and the ideas of specificity of content and function and the 'sidedness' of membranes. How does the cell maintain the proper 'side' of the membrane exposed to the cytosol in all organelles (including the PM)?(1)

c) Outline the mechanisms by which proteins are transported to different membrane-bound compartments in the cell. (2)

d) Compare and contrast N- terminal signal sequences with the 'start transfer sequences' that occur internally within the sequence of membrane proteins. (2)

e) What are the common properties of 'start transfer' and 'stop transfer' sequences? (2)

f) What determines whether the N-terminal end of a protein is in the cytosol or in the ER cisterna? (2).

g) Explain the role of chaperones in protein processing. (2)

h) Many membrane proteins contain a number of membrane crossing domains. How do these proteins get inserted into the membrane? (3)

i) Compare and contrast the mechanism of protein insertion in the ER with nuclear import. Think about the ideas of necessary and sufficient and how they apply to the endomembrane protein targeting system (3).

Topic 5.3 – Vesicle Transport

a) Fill in the following table to compare and contrast vesicle coat formation:

	Secretory			Recycling	Endocytic
	ER→Golgi	TGN→PM	TGN→Lysosome		
Likely cargo?					
Coat Protein?					
Adaptor?					
(Extra hard challenge questions below this)					
GTPase involved?					
Dynamin? (Y/N)					

b) What is the role of clathrin or other coat proteins in the selection of cargo, and the formation of membrane vesicles? (2)

c) How do coat proteins facilitate the process of vesicle formation? (2) What sort of forces need to be overcome to form a vesicle? (3)

d) What are SNAREs and how do they regulate membrane fusion? (2)

Topic 5.4 – The Golgi Apparatus

a) Define the following terms and determine which ones might be synonyms of each other (1):
   - *Cis*-Colgi cisternae
   - *Trans*-Golgi cisternae
   - *Trans*-Golgi Network
   - Golgi lumen
   - Glycoprotein
   - Proteoglycan
   - KDEL Receptor

b) What portion of a membrane glycoprotein is glycosylated (cytosolic, membrane crossing domain, or the portion facing the lumen of the ER)? (1)
c) What is the role of glycolipids in the glycosylation of proteins? (2)
d) How do cells 'know' where to add oligosaccharides to prospective glycoproteins during the glycosylation process? (2)
e) The lumen of the ER contains a number of enzymes that are involved in protein processing. These proteins are retained in the ER lumen, even though most of the material in the lumen is transferred to the Golgi through vesicle transport. How does this happen? (2)
f) Compare and contrast the structure and function of plant and animal Golgi. (2)
g) Sometimes the enzymes of the ER are accidentally transported to the Golgi. How are these proteins recovered and returned to the ER? (3)
h) Explain the central role of the Golgi in the endomembrane system, with respect to protein synthesis, import of material from the cell exterior, vesicle traffic and cellular digestion (3).

Topic 5.5 – Exocytosis and the Lysosomal Pathway

a) Describe the structure and function of the lysosome. How does it fit into the rest of the endomembrane system? (1)
b) Compare and contrast the regulated and constitutive secretory pathways with regard to the types of material carried and the regulation of secretion. (2).
c) Explain how a protein can continue to be processed even after it gets incorporated into a secretory vesicle. (2)
d) Trace a molecule of a lysosomal protein from its point of synthesis on a ribosome in the cytosol, to its final destination in a lysosome. Include everything that must happen to it for successful targeting to the lysosome, and where each of those things happen in the cell. Be as specific as you can. (2)
e) Explain how the mannoe-6-phosphate receptor is recycled. (2)
f) What determines whether a protein enters the regulated or the constitutive secretory pathway? (3)

Topic 5.6 – Endocytosis

a) What is the difference between early and late endosomes? (1)
b) Define receptor-mediated endocytosis and describe the steps. Be as specific as you can be. (2)
c) In both endocytosis and secretion, proteins are sorted into vesicles destined for different target compartments. Where does sorting occur in each pathway? What are the similarities and the differences? (2)
d) How does an early endosome 'become' a late endosome, and then a lysosome? (3)
e) Do all receptors that get endocytosed get recycled back to the plasma membrane? What happens to the ones that don't? (3)

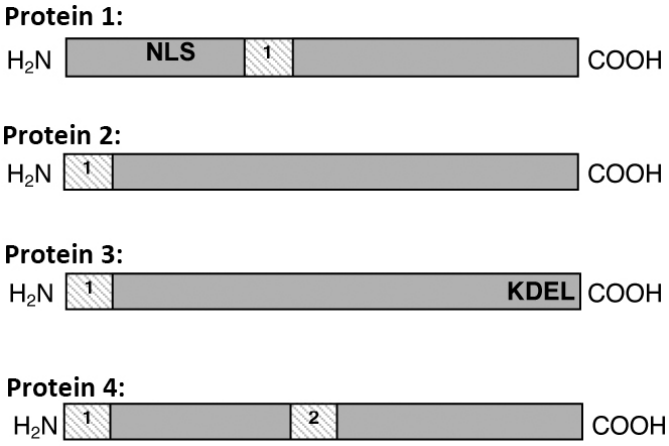
Part 2. Data Analysis and Thought Problems.

These questions will require you to apply your knowledge of the material in ways that may be different from what you've done before. They are meant to test your understanding of the material, rather than your knowledge of it. These questions require critical thinking and problem solving skills that you will practice in tutorial. **They are also the kinds of questions you can expect to see on the exams.** We include some sample problems with solutions, so that you can see what a 'good' answer might look like.

Sample Problems. Answers to these questions are in an adjacent file on Connect.

Sample Question 1:

A. Four proteins are represented schematically to the left. The numbered boxes represent sequences of hydrophobic amino acids. Predict the destination of each protein.



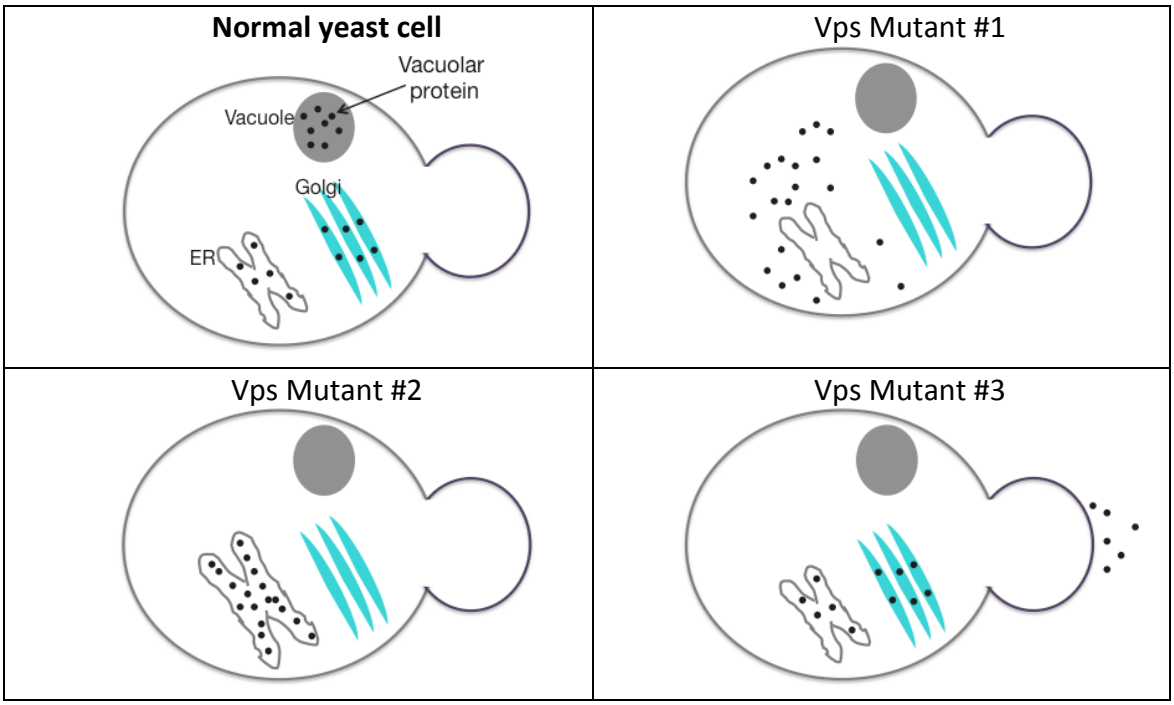
B. For proteins 1 and 4 in part A, draw a fully labeled diagram indicating the orientation of the protein in the membrane.

Sample Question 2:

Yeast vacuoles are acidic organelles that are functionally similar to mammalian lysosomes. To identify the machinery that regulates vacuolar protein sorting and transport, scientists have used yeast cells with mutations in vacuolar protein sorting (therefore called *vps* mutants). Below are diagrams of a normal yeast cell and 3 *vps* mutant yeast cells.

A. For each mutant shown below, explain what cellular process is most likely defective.

B. Given what you know about the mammalian lysosomal pathway, name one protein that might be mutated/defective in each mutant.



Practice Problems.
These questions have no answer key, to encourage you to work with your peers and discuss them. Answers keys can fool you into thinking you understand the question simply because it makes sense when you read it. Being able to explain your reasoning to someone else is a much better test of your understanding and critical thinking skills. We will cover many of these questions in class and tutorial. Trying them on your own followed by discussing them with your peers will ensure that you are well prepared for the exams. Note that the problems are roughly divided by topic, but you should expect to use knowledge from other parts of the course as well.

Topic 5.1 & 5.2 – The Endomembrane System and Protein Targeting to the ER

Q5.2.1

Where would you expect to find a genetically engineered fluorescent protein that contains the following signals? How are these signals used by the cell to maintain these proteins in their proper location?

a) An N-terminal signal sequence, an internal STOP and an NLS.
b) Only a KDEL sequence
c) An N-Terminal signal sequence, and a protein sequence indicating the addition of an M6P oligosaccharide.
d) No sorting signals at all.

Q5.2.2

a) Membrane-bound and free ribosomes, which are structurally and functionally identical, differ only in the proteins that they are making at a particular time. Explain.
b) Make a list of which compartments in the cell use free ribosomes to synthesize their proteins, and which ones are synthesized via membrane-bound ribosomes.

5.2.3

Proteins undergo different processing and transport, based on the cues within their primary sequence. For example, a cytosolic protein and a transmembrane protein in the plasma membrane will have different cues in their primary sequence that will result in differences in translation, processing and in their final destination.

List as many differences as you can think of that you would expect to observe when comparing a cytosolic protein versus a transmembrane protein in the plasma membrane.

5.2.4

Jellyfish make a protein that is fluorescent, called **Green Fluorescent Protein, or GFP**. GFP is often used by researchers to determine the cellular localization of a protein of interest. When the gene of GFP is introduced into cells on its own (i.e. not attached to another protein), GFP is expressed and localized in the cytoplasm of the cell.

What specific sorting signal(s) would you have to add to the GFP protein to produce a protein that has the following cellular destination:

a) Secreted (i.e. released into the extracellular space)
b) Maintained in the lumen of the ER
c) Resides in the lysosome

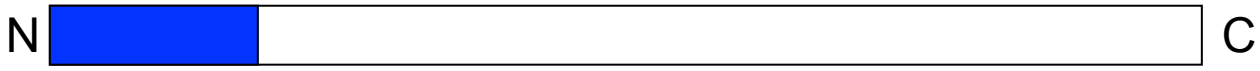
5.2.5

You are working with a protein that is **normally found in the cytosol of the cell**. You wish to change its cellular location by modifying its primary sequence. Would the cellular location change? If yes, explain how.

a) You add 18-20 hydrophobic amino acids to the amino-terminal end.
b) You add 18-20 hydrophobic amino acids in the middle of the primary sequence.

5.2.6

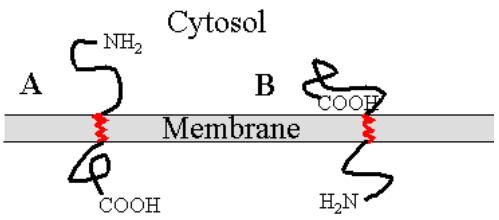
Here is a domain map for a protein. The blue square represents hydrophobic amino acids.



a) Where would this protein end up?
b) What would happen if the hydrophobic amino acids were moved to the centre of this protein?
c) How many different ways can you alter its destination within the endomembrane syste, by adding one additional sorting signal to this protein?

5.2.7

a) Make a map showing the location of signal sequences, start transfer sequences and stop transfer sequences in protein A and protein B below that end up inserted in the membrane as shown.



Protein A

Protein B

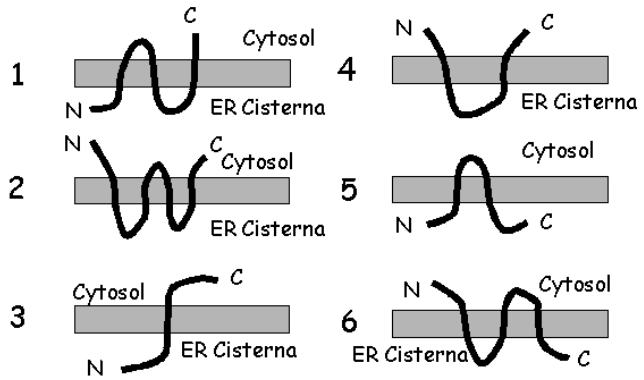
b) Make a map for the arrangement of signal sequences, start transfer sequences and stop transfer sequences for a double pass protein with the N terminal end...

- In the cytosol
- On the extracellular side of the plasma membrane

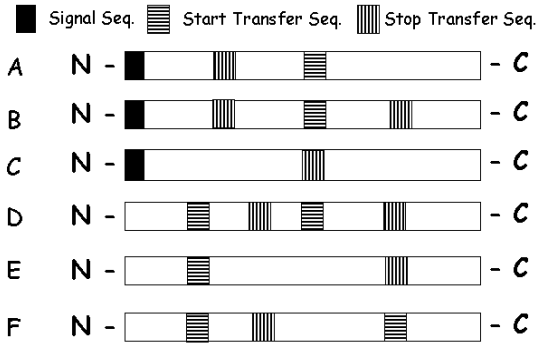
5.2.8

Match the following arrangements of proteins in the membrane with the appropriate polypeptide domain maps below:

Protein Maps

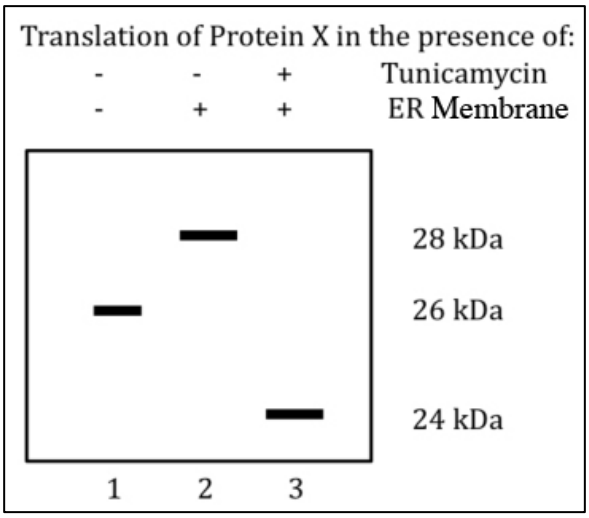


Domain Maps



Q5.2.9

In vitro protein synthesizing systems, containing free amino acids, mRNA, and cytosolic extracts, can be used to study protein synthesis. The cytosolic extracts provide ribosomes and all cellular proteins and factors necessary for protein synthesis.



The gel on the right is a protein gel showing the results of experiments conducted using this *in vitro* system, the mRNA of a glycoprotein (protein X) and the following experimental conditions:

Lane 1: protein X was synthesized in the absence of ER membrane preparations or tunicamycin (see notes below).
Lane 2: protein X was synthesized in the presence of ER membrane preparations in the medium and in the absence of tunicamycin.
Lane 3: protein X was synthesized in the presence of both ER membrane preparations and tunicamycin.

Notes:

1. For the experimental samples analyzed in Lanes 2 and 3, <u>all</u> co-factors necessary for co- translational transport are provided with the ER membrane preparations.
2. Tunicamycin is an inhibitor of glycosylation and can cross the ER membrane.

Questions:

a) What observations can you make about the proteins in each treatment?
b) Explain the differences in lane 1 versus lane 2?
c) Explain the differences in lanes 1 and 2 versus lane 3?

5.2.10

Cystic Fibrosis is a genetic disorder in which patients lack a Cl- channel (known as the CFTR protein) in epithelial cells that is important in the formation of sweat, mucus and digestive juices. As a result of the loss of this channel, patients suffer a variety of symptoms, including difficulty in digesting fats, vitamin deficiencies due to loss of pancreatic function, and progressive loss of lung function.

Genetically, there are a variety of mutations in the CFTR gene that result in cystic fibrosis. Based on how the mutation manifests in cells, we can learn something important about where in the portein the mutation might be found. Can you make some predictions based on the evidence below?
(Note that these mutations may require you to use your knowledge from other topics in this course).

a) The most common mutations result in lack of the channel protein in membrane. Normal amounts of protein are produced. The mutant protein enters the ER properly, but is then degraded by the cell so it never reaches the surface.
b) Alternately, the protein does not get degraded by the cell, but is found in large amounts in the trans Golgi Network.
c) In another mutation the protein produced is extremely truncated, resulting in a protein that is nothing like the original CFTR protein.
d) A third mutation has the CFTR protein being produced and secreted normally, but then the protein ends up in the endosome.

5.2.11

Co-translational insertion of proteins into the ER can be studied in a cell-free system that uses small enclosed segments of rough ER (called microsomes) in a test tube. Researchers studied whether a protein was undergoing co-translational insertion into the ER. The mRNA was allowed to translate in the cell-free system, in the presence or absence of microsomes, treated in 4 different ways:

- No treatment
- Addition of a protease to the cell-free system
- Addition of a protease and detergent to the cell-free system
- Addition of Endo H (removes polysaccharides) in a way that allows Endo H to pass through the microsomal membrane.

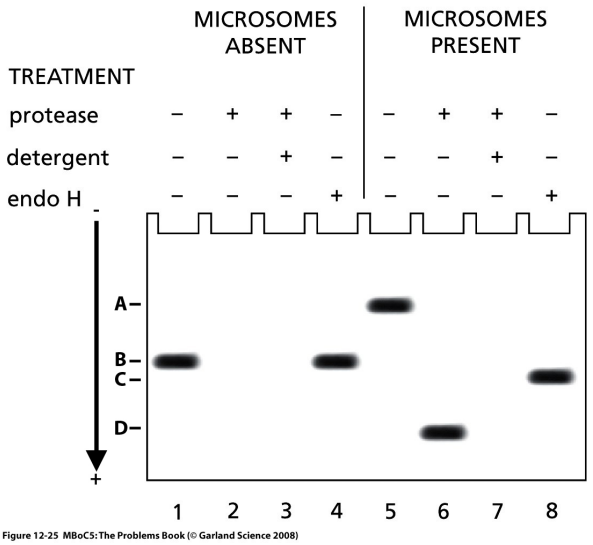


Figure 12-25 MBoC5: The Problems Book (© Garland Science 2008)

The resulting proteins were then subjected to SDS-PAGE, and the results are shown in the image above.

a) What do the data show and mean?
b) Co-translational insertion of proteins into the rER microsomes can be judged by several criteria, including:
    - Whether the newly synthesized proteins are protected from proteases added to the test tube,
    - Whether the newly synthesized proteins are glycosylated by ER enzymes,
    - Whether the signal peptide is cleaved by ER-localized signal peptidase.
c) Using these 3 criteria, draw a conclusion about whether this protein is co-translationally inserted into the rER microsomes or not. Explain your reasoning, and indicate which lane(s) helped you to draw these conclusions.
d) Is this protein an integral membrane protein? How do you know?

5.2.12

Several drug companies are experimenting with the use of peptides (i.e. very small proteins containing only 15-20 amino acids) that specifically interfere with biological process(es). You develop a peptide that binds to the SRP.

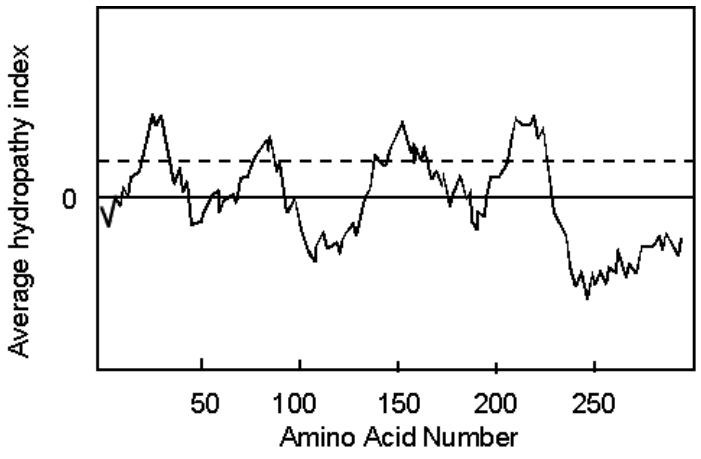
a) Explain what you would expect to happen inside the cell if you were to treat cells with high concentrations of this peptide.
b) List all of the cellular processes that would be directly disrupted by this peptide.

5.2.13

The figure on the left is an average hydropathy plot for a protein. The N-terminal end is amino acid number 1.

Given that amino acid 1 represents the first codon of the open reading frame for this protein, sketch the following:

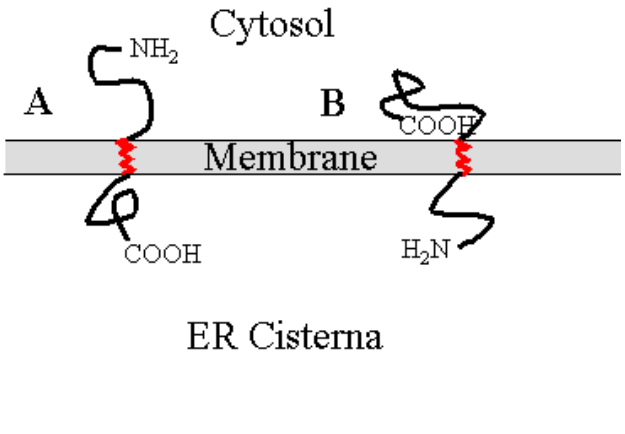
a) The domain map for the protein.
b) The arrangement of this protein in the membrane. Be sure to label the N-terminal and C-terminal ends of the protein, and the cytosol and lumen sides of the membrane.

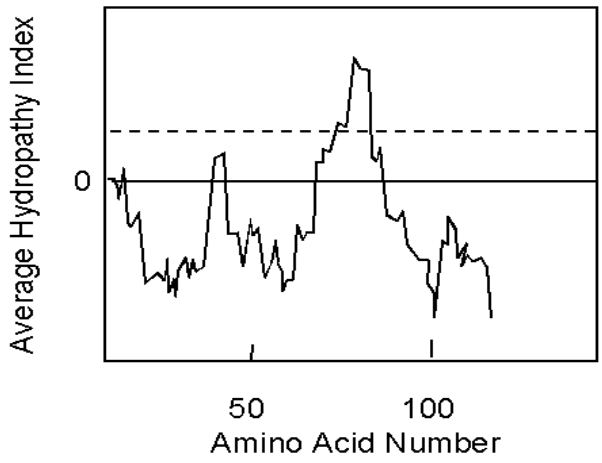


5.2.14

Match the hydropathy plot on the left to the corresponding protein on the right (A or B).

The hydropathy plot matches protein A. Describe how you would alter the hydropathy plot for it to correspond to the one you didn't pick in part a).



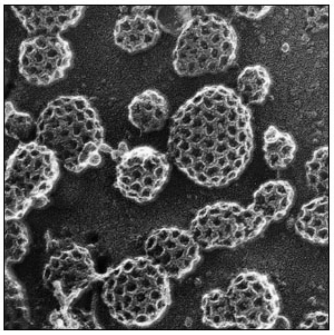


Topic 5.3 – Vesicle Transport

5.3.1

The micrograph on the right shows the formation of clathrin-coated vesicles.

a. List all of places where you find clathrin-coated vesicles forming in the cell.
b. What processes do clathrin-coated vesicles mediate?



5.3.2

A temperature-sensitive mutant with defective dynamin is examined at the restrictive temperature by TEM, and is found to have the structures shown below along the plasma membrane. Explain these results.

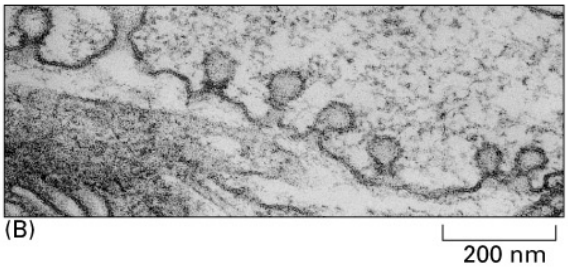
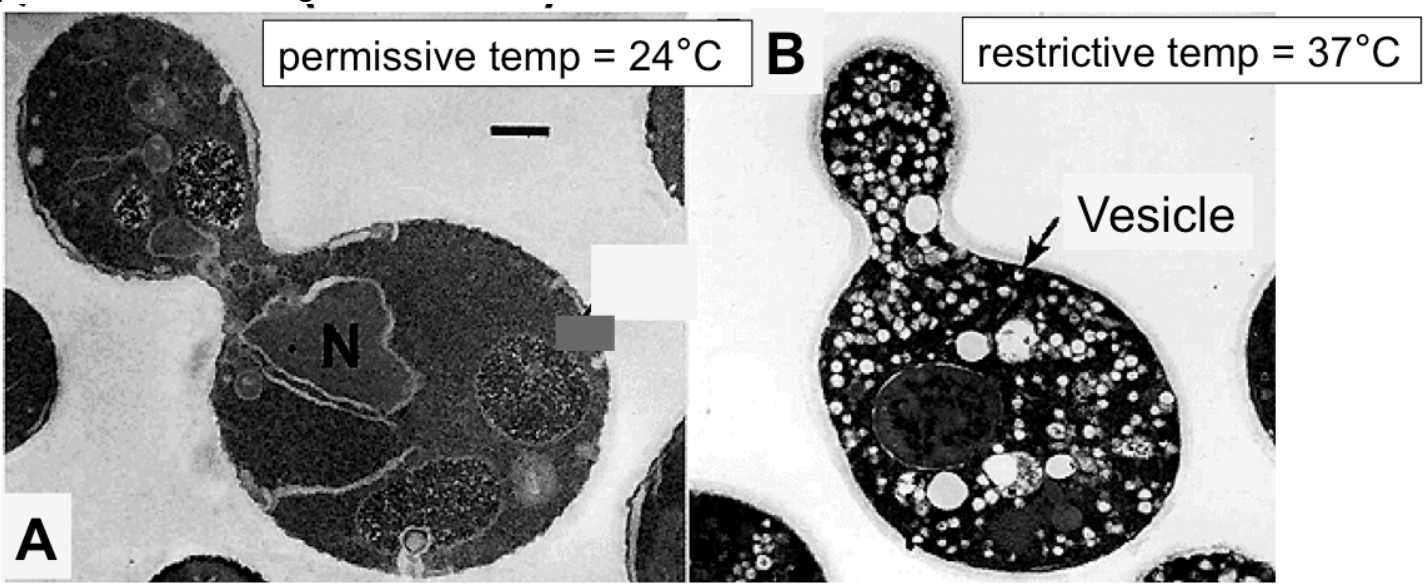


Figure 13–9. Molecular Biology of the Cell, 4th Edition.

5.3.3

In his Nobel Prize winning work, Randy Sheckman and colleagues produced a series of temperature sensitive yeast mutants. The images below are from one such mutant.



a) Which image shows the control? What is it controlling for?
b) What does the data show?
c) What conclusions can you draw about the role of the mutated protein in this case?
d) There are limitations to what this data can tell you about what's happening in the cell at restrictive temperature? So what CAN'T this data tell you?

5.3.4

Explain the following experimental results:

a) A mutant cell in which the COP2 proteins have lost ability to interact with cargoes, all plasma membrane proteins are mislocalized to the ER.
b) A population of animal cells is engineered to express a secreted protein fused to GFP. When these cells are treated with colchicine, the fluorescence accumulates in COPII-coated vesicles.

5.3.5

Consider the v-SNAREs that directly transport vesicles from the trans Golgi network (TGN) to the plasma membrane. They, like all other v-SNAREs, are membrane proteins that are integrated into the membrane of the ER during their biosynthesis, and are then transported by vesicles to their destination. Thus, transport vesicles budding from the ER contain at least two kinds of v-SNAREs - those that target the vesicles to the cis Golgi and those that are in transit to the TGN to be packaged into different transport vesicles (as cargo) destined for the plasma membrane.

a) Why do you suppose this might be a problem for the cell?
b) How do you suppose the cell might solve this problem?

5.3.6

How can it possibly be true that complementary pairs of specific SNAREs uniquely mark vesicles and their target membranes? After vesicle fusion, the target membrane will contain a mixture of t-SNAREs and v-SNAREs. Initially, these SNAREs will be tightly bound to one another, but another protein, NSF, can pry them apart, thus reactivating them. What do you suppose prevents target membranes from accumulating a population of v-SNAREs equal to or greater than their population of t-SNAREs?

5.3.7

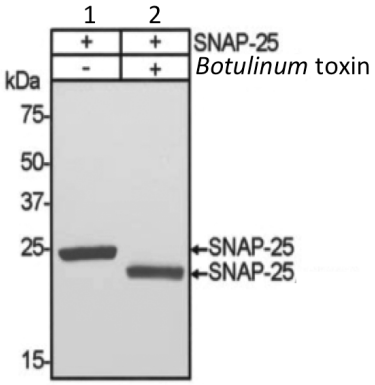
Botox® is a protease from the bacterium *Clostridium botulinum*. Low concentrations of this toxin are commonly used to eliminate wrinkles, because it prevents the secretion of acetylcholine (a neurotransmitter), thus blocking nerve impulses to muscles underneath.

Rickman *et al*. (2004; JBC 279:644) investigated whether the *botulinum* toxin had any direct effects on the SNAREs involved in release of acetylcholine. The t-SNARE, SNAP-25, forms a dimer with another t-SNARE that then binds to the v-SNARE to allow vesicle fusion. In all cases the scale bar shown is 5 µm.

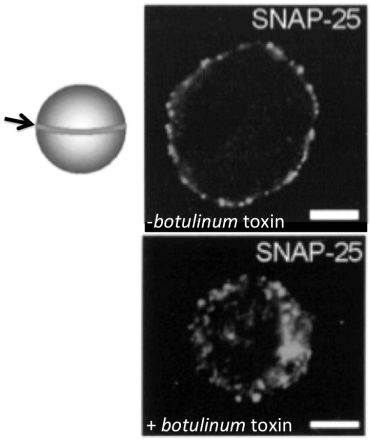
a) Panel A shows an SDS-PAGE protein gel of isolated SNAP-25 protein. Describe what these data show, and draw a conclusion about the effects of the toxin on SNAP-25.

b) Panel B shows the localization of SNAP-25 in the cell with and without the *botulinum* toxin present. The grey sphere in the panel represents a whole cell, with a dark line and an arrow indicating the plane of section of these fluorescent images. Where is SNAP-25 localized in this cell when the toxin is not present? Does the localization change when the toxin is introduced? Explain your assessment.

Panel A



Panel B



c) Based on the data given in all panels, can you hypothesize a model for how the *botulinum* toxin works to prevent the release of acetylcholine to induce paralysis?

Topic 5.4 – The Golgi Apparatus

5.4.1

d) Are proteins in the nucleolus glycosylated? Justify your answer.
e) List all of the places in the cell that you would expect to find glycosylated proteins

5.4.2

A graduate student was asked by her supervisor to learn about a specific protein. She added a GFP-tag to the protein and used fluorescence microscopy to track it through the cell. The fluorescence was observed first in the ER, and then in the Golgi Apparatus. 12 hours later, the fluorescence was still in the Golgi Apparatus.

a) Why would this protein stay in the Golgi?
b) Describe the kind of localization signals that this protein would need to localize to the Golgi and stay there.

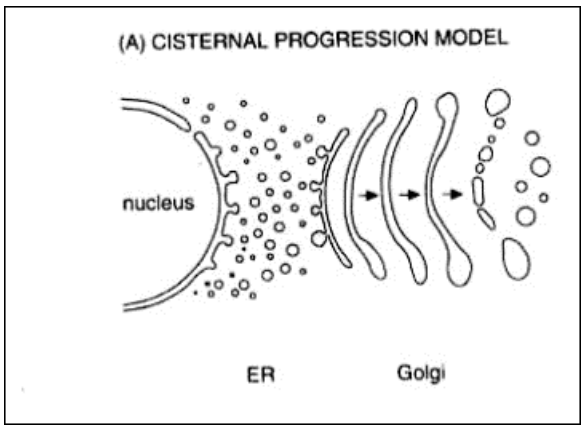
5.4.3

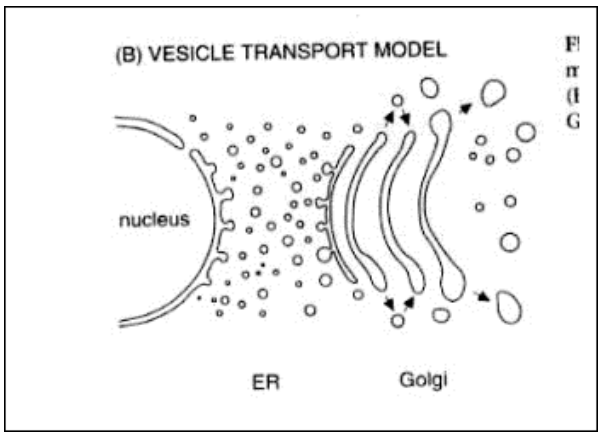
Cells are engineered to express a GFP-tagged lysosomal enzyme. When these cells are treated with tunicamycin, a drug that blocks glycosylation in the ER, the GFP-tagged proteins are secreted.

a) Describe the steps of glycosylation, and identify which step is most likely inhibited by tunicamycin
b) Explain how the addition of tunicamycin could result in lysosomal enzymes being secreted.

5.4.4

Over the years, much work has been done by scientists to determine how cargo moves through the cisternae of the Golgi Apparatus. Many models have been put forward over the years. The Cisternal Maturation Model was one of the first models in the 1960's. It was based on morphological observations of scale synthesis in a number of different types of photosynthetic algal protists. By the 1990s, the generally accepted model was called the Vesicle Transport Model. This was based on some quite clever experiments with animal cells. Drawings to represent these 2 models are shown below:





In the Cisternal Maturation Model (Panel A), new cisternae form continuously as vesicles from the ER coalesce at the *cis* face of the Golgi. Each newly formed cisterna moves through the stack with appropriate modification of its contents, and finally breaks up into transport vesicles at the *trans* face. In the Vesicle Transport Model (Panel B), cisternae remain fixed and the maturing glycoproteins move from the *cis* to the *trans* cisternae in transport vesicles.

One experimental test of these models involved following the path of a protein (called protein A) through the Golgi apparatus. As it moves through the Golgi, Protein A gets glycosylated, just like any other protein. It is normally glycosylated with both:

- Glucose (in the medial compartment of the Golgi) and…
- Galactose (in the trans compartment of the Golgi).

The experiment made use of mutant cells that are defective in the addition of galactose to glycoproteins, and normal cells that had no problem adding galactose to glycoproteins.

In each experiment some Golgi were labeled with radioactive glucose. The cells containing these labeled Golgi were then fused with cells with unlabeled Golgi. As such the hybrid cell that was produced contained functional Golgi of both types (with and without labeled glucose). (For more information, see Rothman et al (1984). J Cell Biol 99: 260-271).

After an hour the cells were dissolved in detergent and the protein being studied was isolated via centrifugation. This protein was separated into two fractions, molecules with attached galactose and molecules without galactose. The fraction with attached galactose was precipitated (pellet), leaving the rest of the protein in solution (supernatant).

The following distribution of label was observed in control and experimental cell combinations:

Cell combination	Fraction of label in pellet (protein with galactose)	Fraction of label in supernatant (protein without galactose)
Labeled wild-type cells fused with non-labeled wild-type cells	85%	15%
Labeled mutant cells fused to non-labeled mutant cells	5%	95%
Labeled mutant cells fused with non-labeled wild-type cells	45%	55%

Based on the information and data above, see if you can answer the following questions (you'll need to make sure that you fully understand what was done before you'll be able to answer these questions):

a) Movement of proteins between which two compartments of the Golgi apparatus is being tested in the experiment? Explain your answer briefly.
b) If proteins moved through the Golgi apparatus by cisternal maturation, explain what you would predict for the results of the experiment? Why?
c) If proteins moved through the Golgi apparatus by vesicle transport, explain what you would predict for the results of the experiment. Why?
d) Explain which model is supported by the results in the table? Why?
e) Do you see any problems with this experiment? Explain.

5.4.5

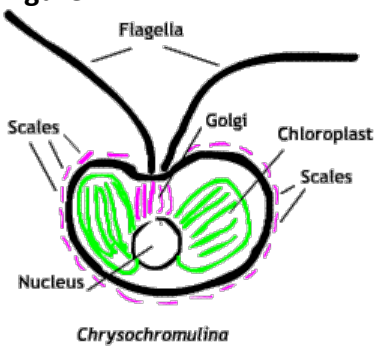
Here is a sample argument outline question for you to practice with. For this question, please write a thesis statement that addresses the statement below, along with 3 supporting arguments, and one piece of evidence that supports each of your arguments. Post your answer on the discussion board so you can work together to compare different approaches to the question, and assess what a good thesis statement + supporting arguments look like.

> *All of the glycoproteins and glycolipids in intracellular membranes have their oligosaccharides facing the lumen side, whereas those in plasma membrane have their oligosaccharides facing the outside of the cell. Explain.*

5.4.6

Many species of algae have large scales on their surfaces. These scales are most commonly made of sugar and/ or protein. As such, they must be produced via the endomembrane system, and then secreted to the cell surface to be assembles. Exactly how this process happens has been studied as a potential way to shed light on the cisternal maturation/ vesicular transport debate.

Figure 1



Chrysochromulina (shown in the diagram on the left) is a small prymnesiophyte alga. The body is covered with several layers of scales. These scales are made within the Golgi apparatus that is located near the bases of the flagella. This study by the pioneer electron microscopist Irene Manton (1968) J. Cell Sci. 2, 265-72 provided some of the first evidence supporting the cisternal maturation model of Golgi Dynamics

The transmission electron micrograph shown in Figure 2 (below) shows the Golgi and the chloroplasts as well as the scales on the surface of the cell (at the left) in section. The inset figure at the lower right shows two isolated scales (metal shadowed whole mount).

Figure 2

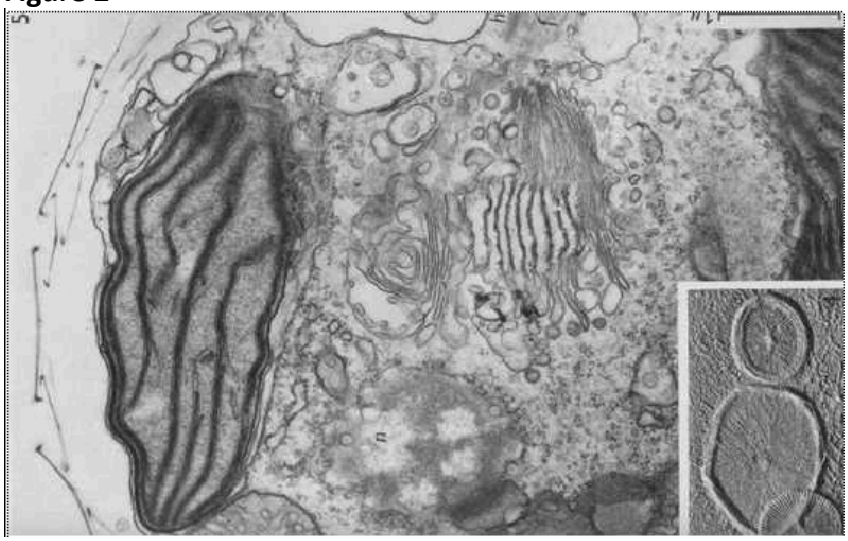
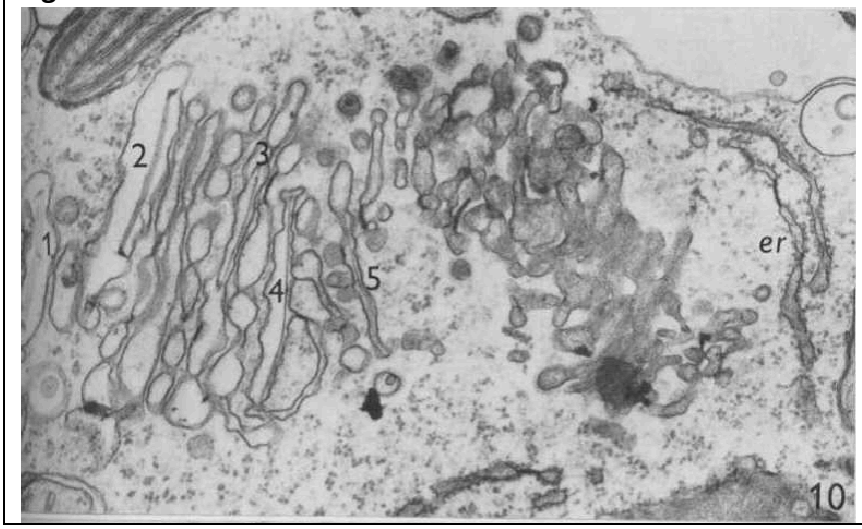


Figure 3



The electron micrograph in Figure 3 shows a section of the Golgi in a Chrysochromulina cell with scales (numbered) at different stages of maturation. #1 is the oldest, and is in a vesicle that is in the process of detaching from the Golgi stack. Scales #2 through #5 are younger with scale #5 being incompletely formed and just barely detectable. Notice that the most immature scale is in the *cis*-most cisterna, and that the most mature is in the *trans*-most cisterna.

Figures 4 & 5 are on the next page. Figure 4 is an example of some more elaborate scales from another alga, *Pyramimonas*. The upper part of the figure (A) shows a section perpendicular to the cell surface. The large scales are on top of a layer of very small scales. The lower part of the figure (B) shows a section through some of the larger scales that is parallel to the cell surface.

Figure 5 show sections through the Golgi apparatus in the prasinophyte alga *Pyramimonas*. The more mature scales are in the *trans*-most cisternae and cisternae that are *cis* to these contain partially

assembled scale material. From Moestrup & Walne (1979) J. Cell Sci. 36, 437-59.

Figure 4

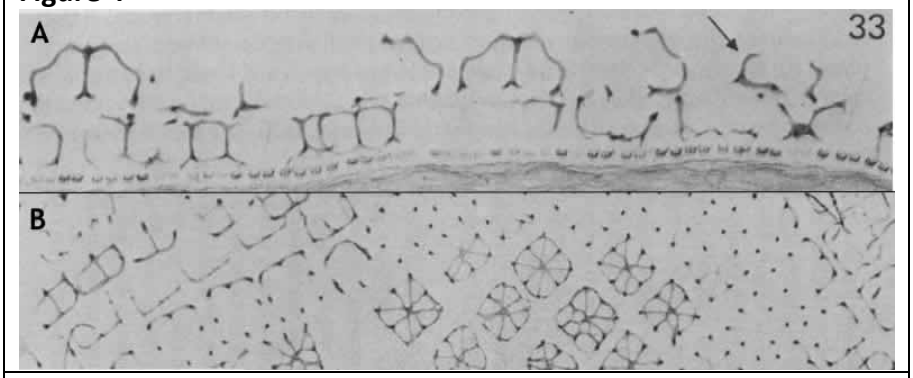
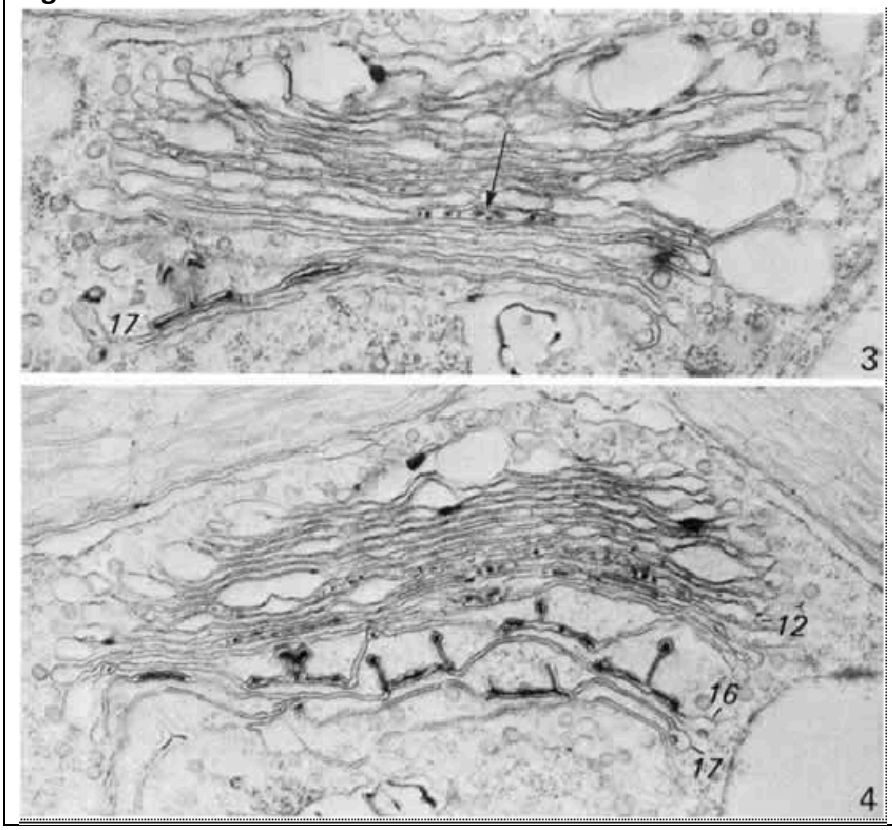


Figure 5



Questions:

a) How does scale synthesis in these algae supports the Cisternal Maturation Model of Golgi dynamics? Explain.

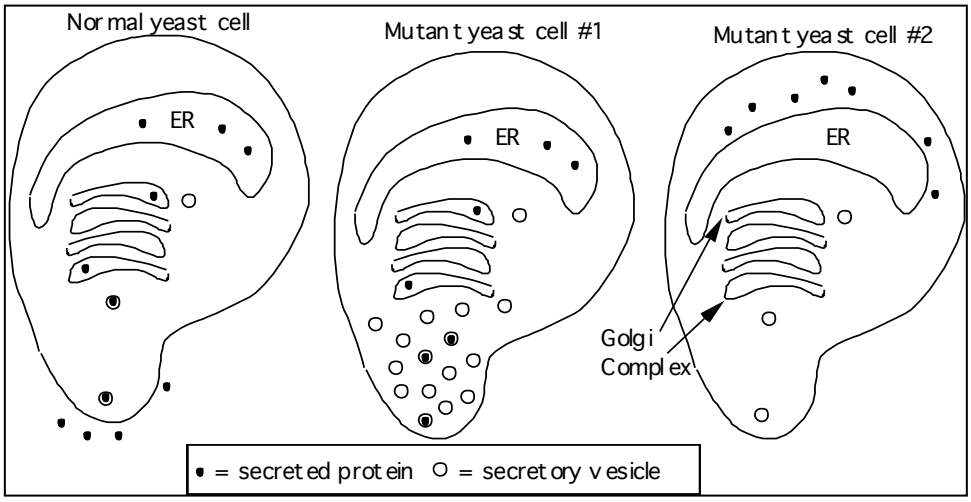
b) Is there any evidence from this data on the vesicle transport model? Explain.

c) Analysis of Golgi traffic has shown that most protein traffic takes 5-10 minutes to pass through from *cis* to *trans*, however these scales can take an hour or more to pass through. Why might that be?

Topic 5.5 – Exocytosis and the Lysosomal Pathway

5.5.1

Normal yeast cells and yeast secretory mutants were characterized using labeled secretory proteins from a variety of genes (shown in black). The results are summarized in these drawings.

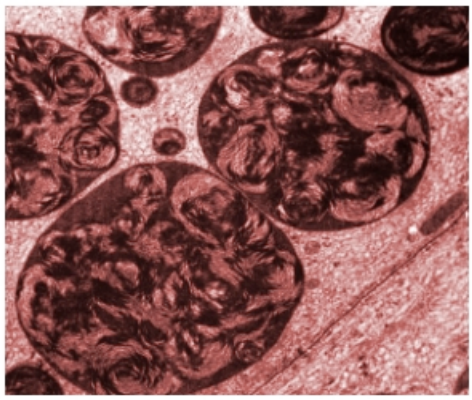


For each mutant:

a) List all of the possible mutations that you could think of that would result in this phenotype.
b) For each of the possible mutations listed about, determine whether it would affect a single specific protein (i.e. produced from a single gene), or all proteins that are being secreted. Any mutations that will only affect a single type of protein should be crossed off your list of potential secretory mutants.
c) Explain how each of the mutations left on your list would result in the phenotype shown above.
d) Consider the limitations of this data. What CAN'T this data tell you?

5.5.2

The TEM image below is of the cells of an 11-year-old boy with a genetic lysosomal storage disease known as Hunter Syndrome. The large structures in this micrograph are lysosomes containing accumulated intact glycosaminoglycans (GAGs), a type of polysaccharide. These lysosomes are much larger than those found in healthy boys of the same age.



a) Explain why the lysosomes in the micrograph look the way that they do?
b) Describe all of the possible cellular mechanisms that could be disrupted in Hunter Syndrome to produce the defective lysosome in the image. Be as specific as you can.

5.5.3

Inclusion-cell (I-Cell) Disease is a lysosomal storage disease. Individuals with this disease accumulate macromolecules within their lysosomes (called inclusion bodies), and lysosomal enzymes can be detected in their blood.

a) What specific cellular mechanism is defective in this disease?
b) How does this defect explain the location of lysosomal enzymes and the formation of inclusion bodies?

5.5.4

The invention of the electron microscope in the 1940s opened up a whole new microscopic world to scientists. They could now see organelles in great detail, and determine the relationship between structure and function much more clearly. One of the scientists at the forefront of this was Dr. George Palade, who won the Noble Prize in 1974 for his work determining the relationship between the different compartments of the endomembrane system. In this problem we will explore the experimental material that was used to determine the order of secretion in the endomembrane system (something we all take for granted now!). The system that Dr. Palade used was that of the acinar cells of the rat pancreas. A diagram and TEM of acinar cells is shown below.

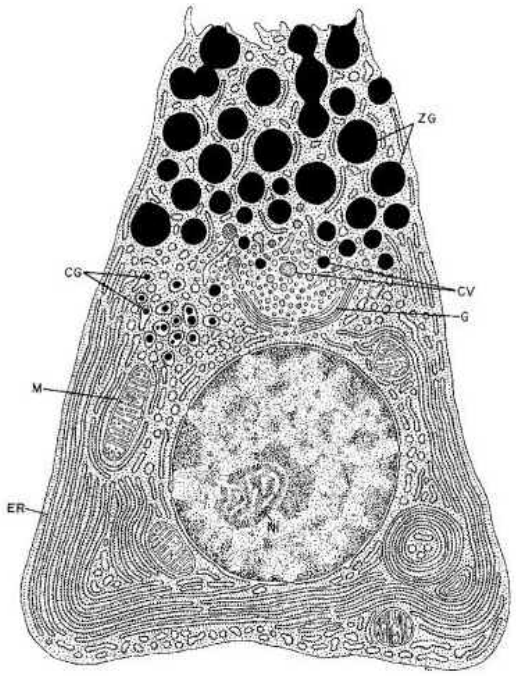


Figure 1: Diagram of acinar cell showing zymogen granules (Black). Zymogen granules (ZG) are a class of dense core secretory granules; ER, G=Golgi, CV=Condensing Vesicle. The cell surface facing the duct is at the top of the diagram.

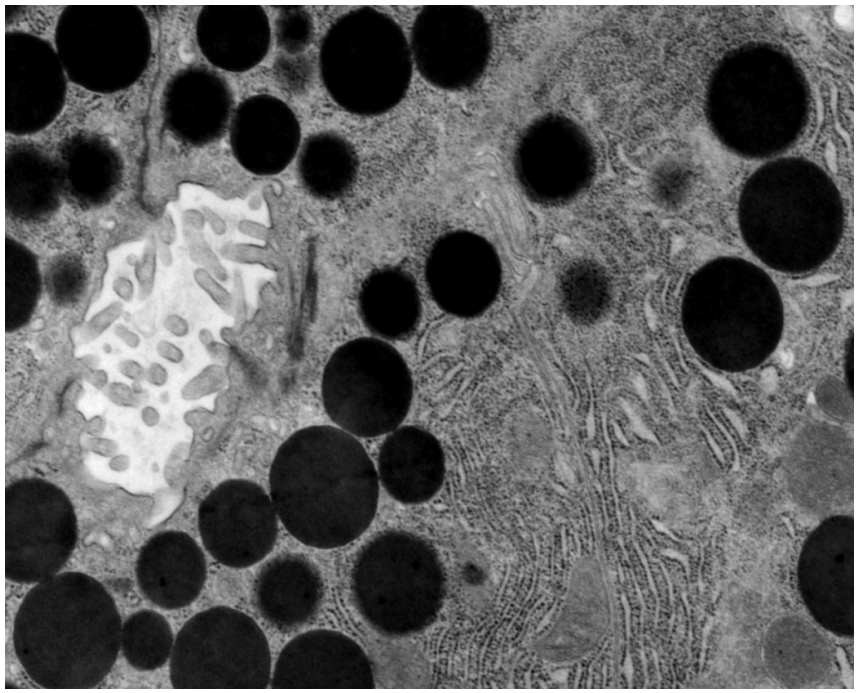


Figure 2: TEM showing part of an acinar cell of the pancreas. Identify as many of the parts as you can see from Figure 1, and determine what part of the cell drawn in 1 is imaged here. Also How many cells can be seen in this image? How do you know?

Experimental Procedure:

In this experiment, a pulse of radioactively (3H)-labeled amino acid is fed to the pancreatic cells for a specific time period, followed by a cold (i.e. unlabeled amino acid) chase of varying time frames.

- At the end of each chase time frame, the cells are fixed, embedded in resin and sectioned.
- The sections are prepared for autoradiography.
- Analysis of pictures of successive stages shows the progression of the protein through several organelles, as shown in the images on the next page.

Questions:

a) Put the 4 images on the next page in the correct order to reflect how the proteins move through the endomembrane system.
b) How does the label move through the cell? How does the data support your conclusion?
c) If a slice of pancreatic tissue had been incubated in ^{3}H-leucine continuously for 2 hr, where would you expect to find incorporated radioactivity in acinar cells?
d) Would you expect the properties of the cisternal side of the Golgi membranes to be more similar to the extracellular or to the cytosolic side of the plasma membrane? Why?

Image A

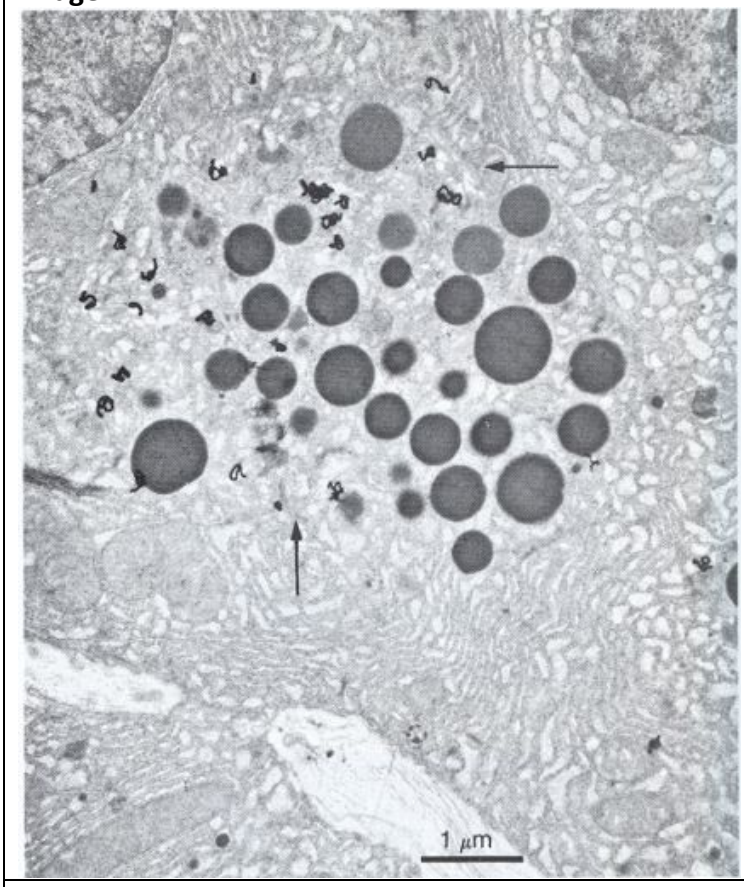


Image B

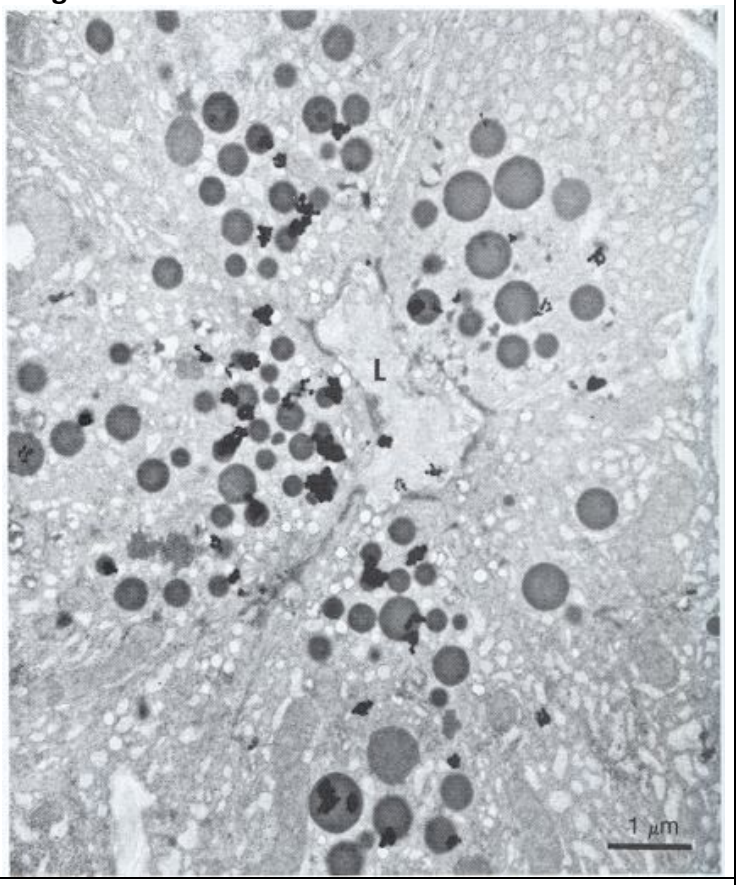


Image C

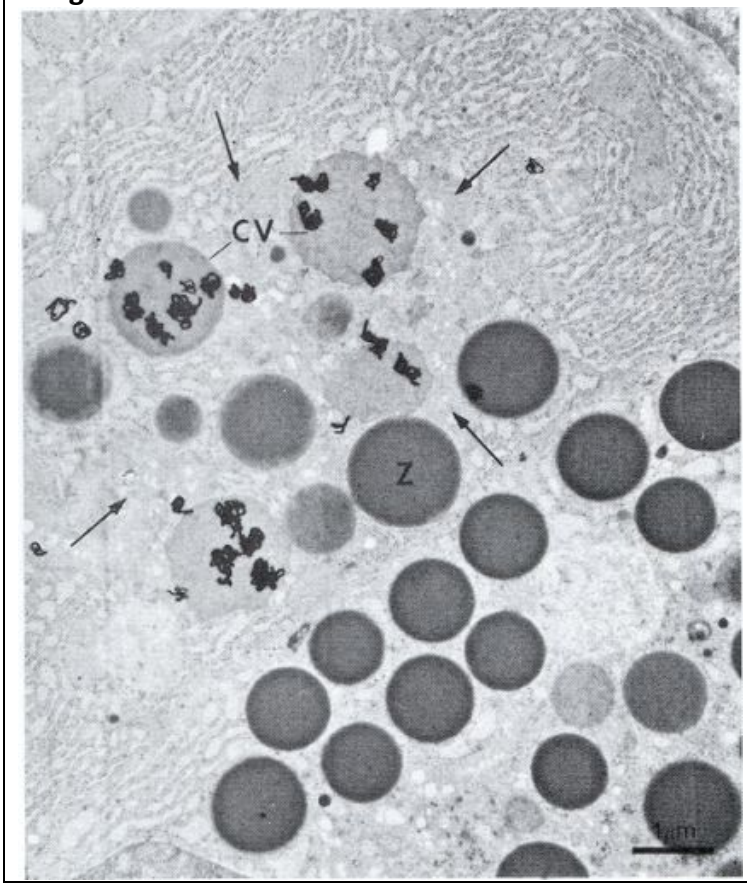
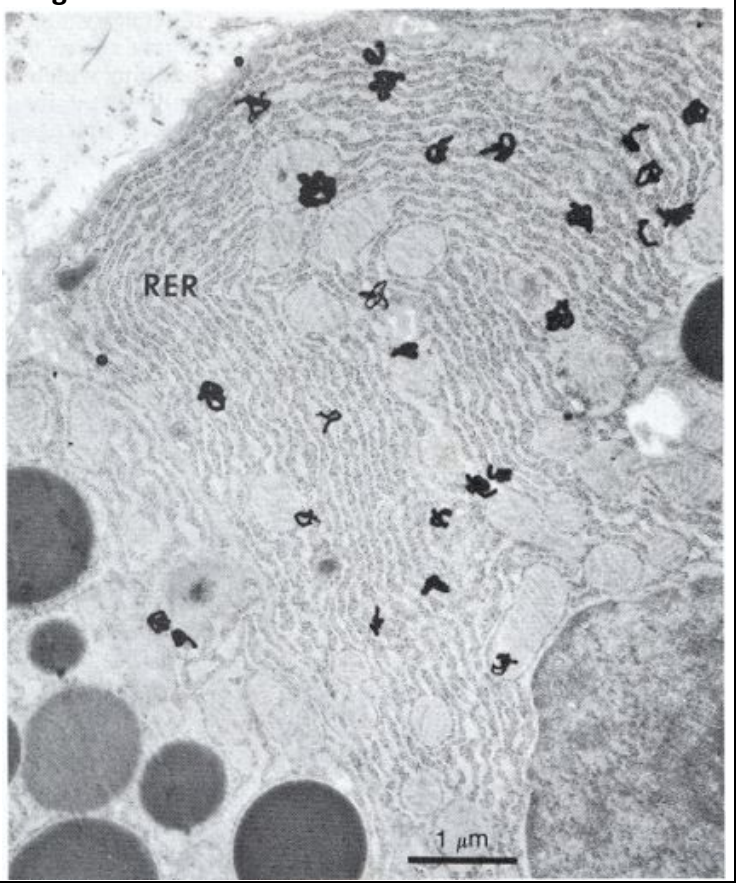


Image D



5.5.5

Insulin is a peptide hormone that is secreted by beta cells of the pancreas into the blood. Figure 1 below shows two dense core secretory granules. The one at the left is ready to be secreted and the one on the right is in the act of being secreted; the vesicle containing the granule has fused with the plasma membrane. Figure 2 shows two domain maps of the insulin protein as it is synthesized. The upper one is the protein right after it has entered the endomebrane system. The disulfide bridges are added during the early stages of the processing of the protein. The final protein that is secreted (lower image, Fig 2) consists only of two short polypeptide chains joined by the disulfide bridges. The entire central part of the original molecule is missing.

Figure 1

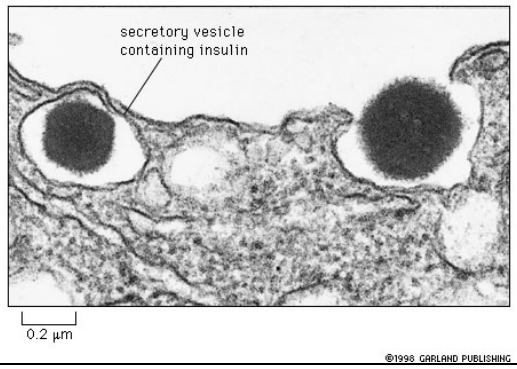
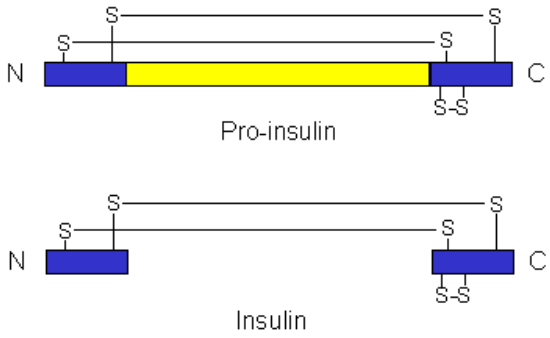


Figure 2



This is an example of processing of a protein by proteolytic cleavage. This occurs commonly in the case of many secreted proteins. As proteolytic processing can theoretically happen at any point during (or after) secretion, scientists often as the question, "When and where does cleavage of the molecule occur?" Understanding how insulin is produced has been extremely important for developing therapies to insulin-related illnesses, such as diabetes.

Here is an experiment that was done to address the question of where cleavage of the insulin molecule occurs. This experiment uses gold-labeled antibodies. Small colloidal gold particles are attached to antibody molecules. These little gold particles show up in electron micrographs as tiny black dots. In each of the images in this table, a different primary antibody was used, resulting in a different protein being labeled with gold.

Figure 3a: Pro-insulin antibody

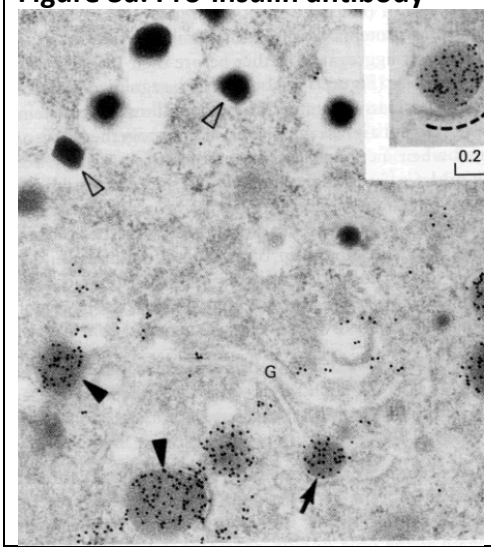
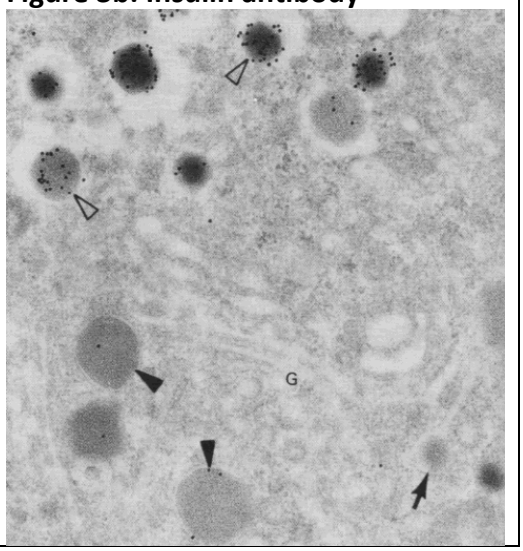


Figure 3b: Insulin antibody



The outlined arrowheads (triangles) point to zymogen granules, whereas the black arrowheads point to condensing vesicles. The black arrow points to a forming vesicles that is still attached to the Golgi (G).

Questions related to this experiment are on the next page.

Being able to identify the small black dots of gold (and understand what they are labeling) is the first step to being able to interpret the results of this experiment. If you are having trouble understanding how antibodies can be used in this context, go to Resources> Study Aids> Background Information> Antibodies and their uses in Biology.

Questions:

a) Can you interpret this data? What is being labeled in each of the images?
b) Do the results of the immunolabeling experiment shown above give you information that answers the question being asked? What conclusions can you draw about where and when proteolytic cleavage of insulin occurs?
c) The results shown here are missing a very important component of a good experiment. What could you add to this experiment to fix it?

5.5.6

Lysosomal enzymes (e.g. acid phosphatase) are produced like other proteins and targeted to lysosomes. They interact with proteins that have been endocytosed and directed to the lysosome for digestion.

Questions:

a) Trace a molecule of the lysosomal enzyme acid phosphatase from its origin on a ribosome in the cytosol until it is finally in place in a secondary lysosome. List in order all of the major events and processes.
b) Trace a molecule of mannose-6 phosphate receptor from initiation of its synthesis on a ribosome in the cytosol until it has been recycled to the Golgi apparatus. List in order all of the major events and processes.
c) If a lysosomal membrane broke down and the lysosomal enzymes found themselves in the cytosol, would they damage the cytosolic proteins? Why or why not?
d) Cells are experimentally engineered to express a GFP-tagged lysosomal enzyme. When these cells are treated with tunicamycin, a drug that blocks glycosylation in the ER, the GFP-tagged proteins are secreted. Explain this result.
e) In plants and fungi, another compartment is thought to play the role of the lysosome… which one is that?
f) When a lysosome-targeting signal is experimentally added to a protein that is normally cytosolic the protein remains in the cytosol. Explain this result.

Topic 5.6 – Endocytosis

5.6.1

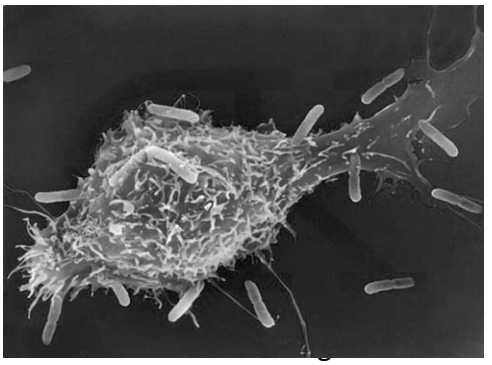
The influenza virus enters human cells via endocytosis. Once the virus is inside the cell, it "escapes" the late endosome by fusing its viral membrane with the endosomal membrane, which releases the viral contents into the cytosol.

a) Where would the virus most likely end up if it doesn't "escape" the late endosome?
b) What would be the end result if the virus doesn't escape?
c) Is there another destination that could be reached from the late endosome? List as many as possible.

5.6.2

You are given a grant proposal to judge on bacterial infections. They show you the image on the right of a white blood cell phagocytosing bacteria and say that they will use this technique to count the number of bacteria in lysosomes inside this cell.

a) What technique are they proposing to use?
b) Can you use this technique to do what they propose? Explain.



5.6.3

Iron (Fe) is an essential metal that enters cells through the Transferrin/Transferrin receptor system. Transferrin, a soluble protein that circulates in the bloodstream, binds to Fe in the blood stream. Transferrin receptor, a plasma membrane protein, binds to transferrin-Fe complex circulating in the blood stream. pH plays a role in the different binding interactions and is described below:

At neutral pH (as in the blood stream), transferrin binds to Fe:	 Transferrin-Fe complex
At acidic pH transferrin does not bind to Fe:	 Unbound Transferrin 
At neutral pH (as in the blood stream), the transferrin receptor is only able to bind to transferrin-Fe complexes:	 Transferrin-Fe bound to receptor
At Acidic pH, the transferrin receptor is able to stay bound to transferrin whether its complexed to Fe or not:	 or 

a) Based on this, describe the pathway that iron takes to enter the cell, and receptor recycling in this system.
b) Describe the localization pattern you would expect to see in a normal cell by immunofluorescence microscopy if you use a fluorescent antibody against the transferrin receptor.
c) Predict how this labeling pattern would change in a mutant cell in which the transferrin receptor cannot bind to adaptin/clathrin complexes? Explain why.
d) Predict how this labeling pattern would change if normal cells were treated with an inhibitor that blocks vesicles from leaving early endosomes? Explain why.

5.6.4

a) Do all molecules that enter early endosomes ultimately reach late endosomes, where they become mixed with newly synthesized acid hydrolases and end up in lysosomes? Why or why not?
b) Explain how an early endosome might become a late endosome, and then a lysosome. What must happen for this transition to occur?