BIOL200 Problem Sets

Content Review Questions & Practice Problems

Updated Summer 2016

Biology 200 Problem Sets

Table of Contents

Introduction to the Problem Sets

Welcome to the BIOL200 problem sets! In these pages are contained the keys to your success in BIOL200! This workbook is designed to help guide your studying and to help you practice the skills that will be required for success on the midterm and final exam.

BIOL200 is a 3-credit course, with 3 hours/ week of class time and 1h/ week of tutorial. The most commonly accepted rule of thumb is that for every hour of class time, you should be spending 2 hours outside of class working on the course. That means we're expecting that for every week of class in BIOL200, **you should be expecting to spend 6-8 hours outside of class working on the course**.

During an average week, 'outside of class' time can be broken up into a few different parts:

1. **The Pre-Readings and Quizzes (~1-2h per week)**
   These quizzes happen on average about once a week, they involved reading material on Connect (based on the pre-reading guides) and then taking a 12-15 question quiz on the material. The goal here is not to learn everything on your own, but rather begin to get some of the basics (i.e. specific jargon, definitions, chemical structures and some of the simpler concepts). If there are things you still don't understand after the pre-reading, that's ok. Simply tell us about it in the last question of the quiz and we'll know to focus on it in class.

2. **Tutorial Preparation (~1h per week)**
   In each week, we will be working through a specific problem from this Problem Set in tutorial, as a way to help you practice your problem-solving skills and prepare for the midterm and final. To prepare for tutorial, we expect you to review the course material for the unit in question, as you will need to know something about cell biology in order to successfully apply your knowledge to the problems. The tutorial problems are identified in the problem set, and you're welcome to take a look at them. You will be given time during tutorial to try them on your own and in groups, so it's perfectly ok if you haven't solved the problem (or even attempted it!) before you get to class.

3. **Personal Study Time (3-5h per week)**
   This is when you work on the course material on your own (or in your study groups), work on the press release assignment, and practice the problem solving skills you will need to succeed on the midterm and final exam. If you don't work on this throughout the term, you can assume that the accumulated time is how many hours you're going to need to put in to prepare for the midterm/ final.

This Problem Set is designed to help you structure your personal study time.

What's in the Problem Sets, and how do they help?

The Problem Set starts with a Table of Contents that is clickable in pdf format, so that you can easily find the unit that you want to work on. We've also broken up this problem set into individual units, and posted them on Connect for you, in case you want to work with a smaller document. The complete document can be downloaded in a single file from the Resources Section of Connect.

In each unit, we have 2 different kinds of questions for you, which have an entirely different purpose for your studying:

1. **Content Review Questions**
    - This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
    - 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.
    - There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.
2. **Practice Problems**
    - The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
    - Most of these questions come from old exams. Some of them are designed to make you think critically, rather than to practice exam-writing.
    - We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
    - Note that the problems are roughly divided by topic, but you should expect to use knowledge from other parts of the course as well.

Where to find more questions on a topic?

In addition to the material in each Unit, we've tried to help identify the interconnected nature of these problems by creating an index for you. The questions have several meta tags associated with them, which helps to identify specific similarities (i.e. common technique or course topic). For example → *(tags: #TEM)*

If you want to find more questions one a topic or technique use the tags, and/ or look the tag/ topic up in the index. This can help you find questions that link content from different units, similar to what you would see on the exam. Unfortunately, this index is not clickable in the pdf, so you'll have to go to those pages manually.

Where to find more help?

If you are having problems with a question, check out the related **Problem Walkthroughs** at the end of the booklet. They are designed not only to show you what the answer to the question is, but to help you understand why its correct, and to help you better understand what a 'good' answer contains. If you need additional help you can post on the discussion board or come to office hours.

What to do if you find an error?

Like all of the documents in this course, they are written by people. Sometimes mistakes happen. If you think you've spotted an error, please don't hesitate to let us know. The easiest way is to contact Dr. Robin directly (biol200@zoology.ubc.ca) as she is the curator of the common course material. You may also contact your instructor if you prefer, who will take note of the information and pass it on to Dr. Robin if necessary.

We do everything that we can to avoid errors, and we rely on you to help us find the ones that we've missed. Future BIOL200 students appreciate your help!

Unit 1: Eukaryotic Cells and Microscopy

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 1.1 – Eukaryotic Cells & Organelles

a) Define the following terms and determine which ones might be synonyms of each other (1):

- Cytoplasm
- Cytosol
- Biological Compartment
- Membrane bound organelle
- Nucleoplasm
- Cell
- Organelle
- Cellular structure

b) Make a list of all of the organelles that you already know, and their functions. Find a friend to compare with and see if your lists match up. (1)

c) List all of the cellular structures that are unique to either plants or animals & their functions. (2)

d) Compare and contrast Prokaryotes and Eukaryotes. (2)

e) Would you consider chloroplasts and mitochondria to be Prokaryotes or Eukaryotes? Why? (3)

Topic 1.2 – Microscopy

a) Compare and contrast the 4 different types of microscopy (Brightfield, fluorescence, transmission electron and scanning electron microscopy). (3) Think about them in the following different parameters:

- Source of images (i.e. photons versus electrons, emitted versus transmitted)
- Path of particle through the microscope to produce image (make a rough sketch)
- Sample preparation
- Major advantages and disadvantages of each kind of microscopy
- Tricks for recognizing micrographs produced by each type of microscopy that you could use on the exam

b) Find a really good website that showcases micrographs from the different types of microscopes and share it on your lecture discussion board. (1) Then look through this (and other) websites to find examples of the different organelles that you listed in the questions above. Try and find examples of images in multiple different types of microscopy. (2)

c) What determines the resolution of a microscope? Why is resolution more important at higher magnifications? (1)

d) Explain how indirect immunofluorescence works. (2)

e) What are the advantages of using Green Fluorescent Protein (GFP) instead of more traditional fluorescent staining techniques? (1) How does it work? (2)

f) What is the primary reason for the resolution limits in light microscopy that electron microscopy was developed to overcome? (2)

g) What is the very smallest thing that scientists have visualized using light microscopy? What about electron microscopy? How does the size of this 'thing' match up with what you know about that microscope's resolution limits? (3)

h) Optical physicists have pushed the boundaries of light microscopy beyond what should be possible, based on what we know about the wavelengths of visible light. See if you can figure out how 'super-resolution' light microscopy works. (*3+, extra challenging question!*)

Practice Problems

- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- 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 1.1 – Eukaryotic Cells & Organelles

Note: Topic 1.1 is an introductory topic, and so the problems in this topic are review, rather than exam-style.

Problem 1.1.1

(tags: #features of eukaryotic cells)

In any course that you will take, it's important to think about the definition of the terms that you are working with. Sometimes considering what does and doesn't fit into your definition is a good way to better understand what you're studying. In this problem we consider the 'Big Picture' and how the material that you will learn about in this course fits into Biology as a whole. Note that you are not looking for a 'correct' answer in this question, but rather a way to explore ideas about cells and their place in Biology.

a) Consider the following terms, and see if you can come up with a definition of each one.
   i. Alive
   ii. Dead
   iii. Cell
   iv. Organelle
   v. Organism
   vi. Virus

b) Then try and think of something that you might consider to be an 'exception' to your definition. Does that mean that your definition needs to be modified? How? Once you have done this on your own, seek out classmates to compare with. See if you can find ways that each other's definition might not quite fit, and then decide if that means your definition needs to be modified.

c) Consider the established cell cultures (e.g. Hela Cells) that are used by scientists to do research. To produce a cell culture, a small subset of cells is taken from a larger multicellular organism, and maintained separately for research purposes. If these cells can survive and proliferate on their own, would you consider them to be an organism? Based on your definition from above? Try and make at least 3 arguments for and against this classification.

d) Finally, consider the case of viruses. Would you consider them to be alive? Why or why not? Try and make at least 3 arguments for and against this classification.

Problem 1.1.2

(tags: #organelle identification)

Consider the following cellular structures. Decide whether you would classify them as an 'organelle' or not, based on your definition from above and justify it. Do any of them fit into any of the other categories from above? Why or why not? What does that tell you about your definitions?

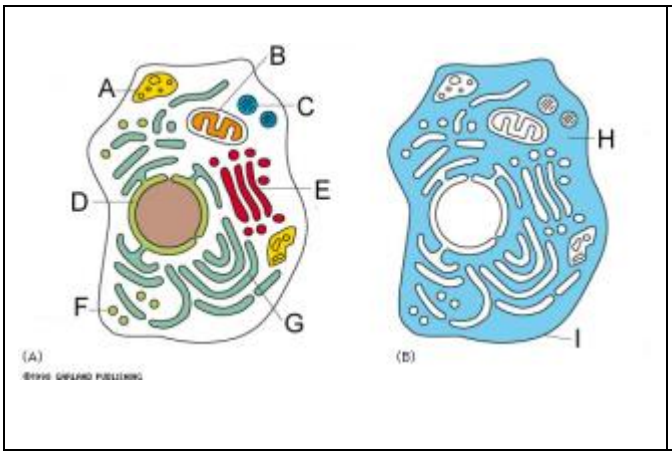
a) Ribosome
b) Chloroplast
c) Plasma Membrane
d) Golgi Apparatus
e) Electron Transport Chain
f) Cilia
g) Plant Cell

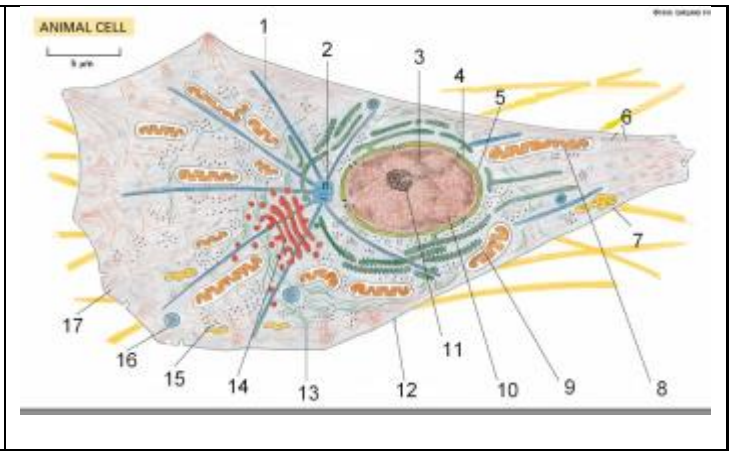
Problem 1.1.3

(tags: #organelle identification)

a) Identify all of the structures that you can in the diagrams below. Then use your textbook to look them up and see if you were right.

b) Compare the two panels of diagram. Which is a more accurate depiction of the cell? What are the advantages and disadvantage of each type or representation of cells for you as a learner?





Topic 1.2 – Microscopy

Since microscopy is so visual, it is difficult to properly display it in .pdf problems. Throughout the course website you will encounter a page for each unit called '**Visualizing the Cell**'. These pages are designed to help you think about the material you're learning about in a visual way, and to practice thinking 3 dimensionally.

We recommend that you continue practicing interpreting microscopy throughout the course by exploring the videos and images on those pages.

Problem 1.2.1 (Walkthrough Available)

(tags: #TEM, #SEM, #brightfield microscopy, #fluorescence microscopy, #organelle identification, #identify microscopy type, #plants, #animals)

Classify each of the images shown into the table below, based on the type of microscopy and the organelles that you can SEE. Be aware that each image may fit into more than one category and not all categories may be present. Marks will be deducted for wrong answers.

Image 1:

Image 2:

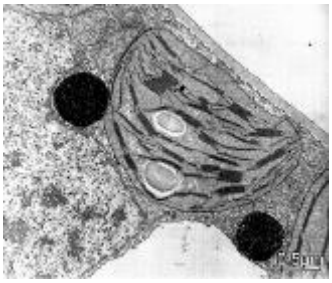


Image 3:

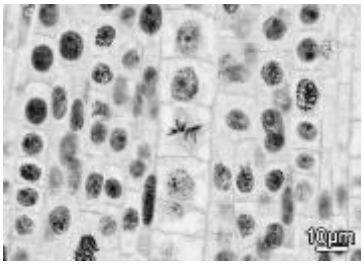
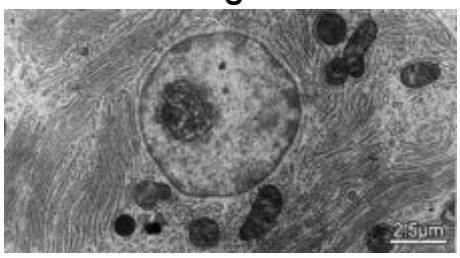


Image 4:



Type of microscopy:	
Brightfield light microscopy	
Fluorescence microscopy	
Transmission electron microscopy	
Scanning electron microscopy	

Organelles:	
Plasma membrane	
Nucleus	
Nucleolus	
Endoplasmic reticulum	
Mitochondria	
Cytosol	
Chloroplast	
Heterochromatin	
Mitotic Chromosome	
Flagella	

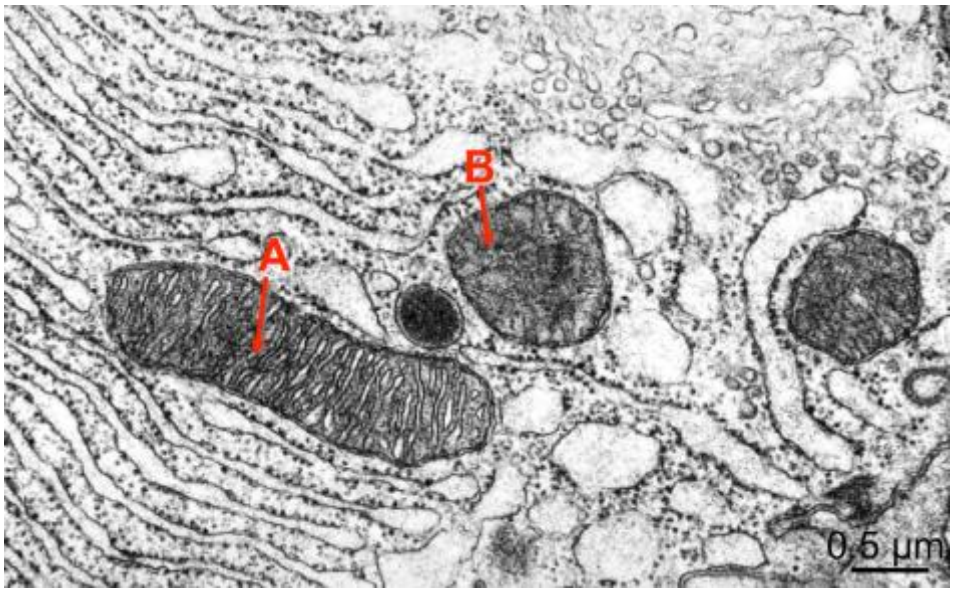
Problem 1.2.2

(tags: #TEM, #organelle identification)

a) The structures labelled **A** and **B** in the image below depict the same organelle. What is it?

b) Why are there such distinct differences in how each of these organelles appear in this image?

c) Draw a diagram of this organelle to help you better understand how A and B could be showing the same organelle, even though they look so different.



Problem 1.2.3

(tags: #TEM, #SEM, #brightfield microscopy, #fluorescence microscopy, #identify type of microscopy, #mitochondria, #chloroplasts, #nucleus, #plants, #animals)

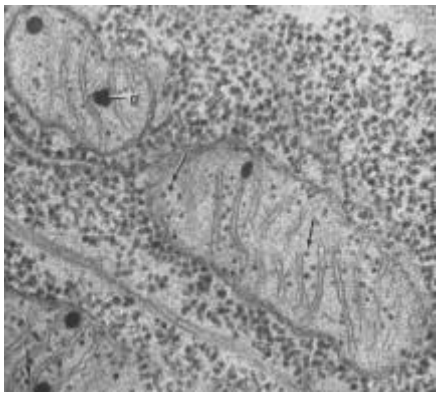
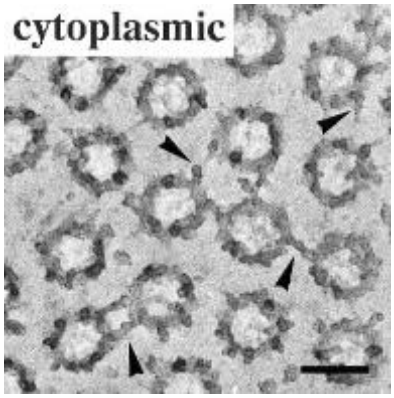
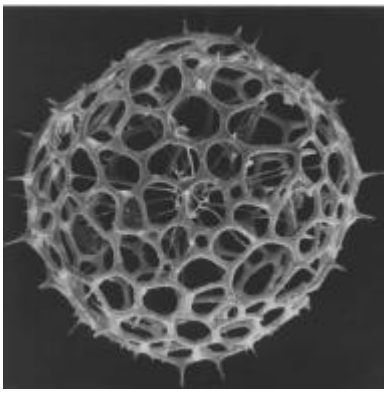
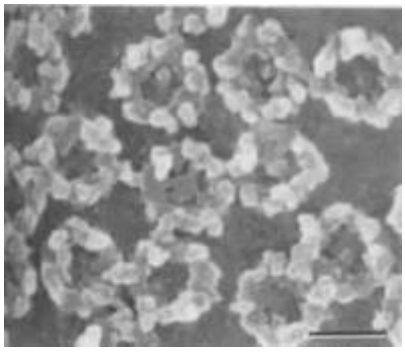
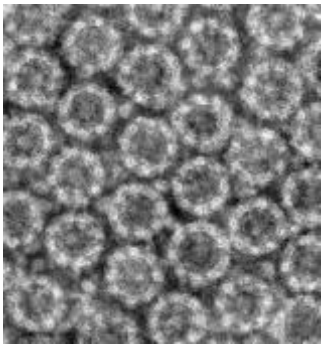
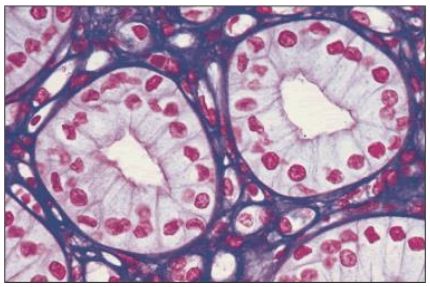
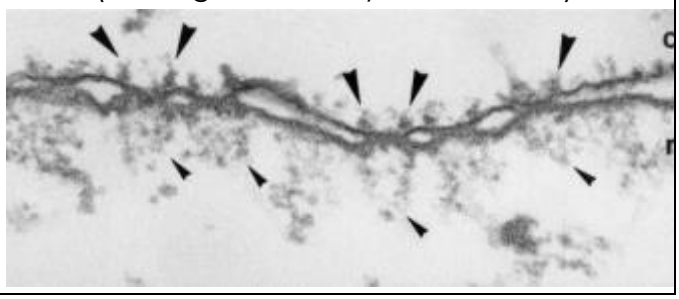
There are a variety of ways a sample can be prepared for microscopy, and still more ways to visualize them. While you will only be tested on the 4 main types of microscopy, it's important to consider how different ways to prepare the same can have different effects on the final image.

Can you identify the microscopic technique used to obtain the images on the next page? Things to consider...

- Type of microscopy
  - Brightfield light microscopy
  - Fluorescence light microscopy
  - Transmission electron microscopy
  - Scanning electron microscopy
- Preparative techniques
  - Thin sections versus whole mount
  - Negative staining
  - Live tissue versus fixed tissue

These images include the following techniques:

- Light microscopy - brightfield illumination
- Light microscopy - confocal fluorescence microscopy
- TEM – thin section (i.e. 'normal')
- TEM - Negative stain
- SEM

1. Mitochondria from a Rat liver Cell 	**2. *Tetrahymena sp.* A ciliated protest stained to show nuclei (red) and a single protein (CDK1) in green.** 
3. Nuclear pore complex, cytosolic surface (more challenging) 	**4. Skeleton of a marine protest of the Phylum Radiolaran, Class Polycystina.** 
5. Nuclear pore complex, cytosolic surface. 	**6. Nuclear pore complex, isolated.** 
7. Cross section of a kidney tubule from a rat (also Fig 1-5 from your textbook) 	**8. Isolated nuclear envelope cross section** (also Figure 15-8 in your textbook) 

Problem 1.2.4

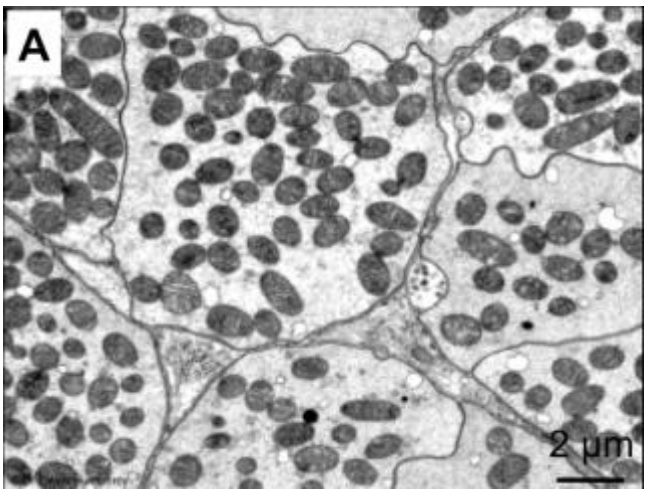
(tags: #TEM, #fluorescence microscopy, #mitochondria, #nucleus, #animals)

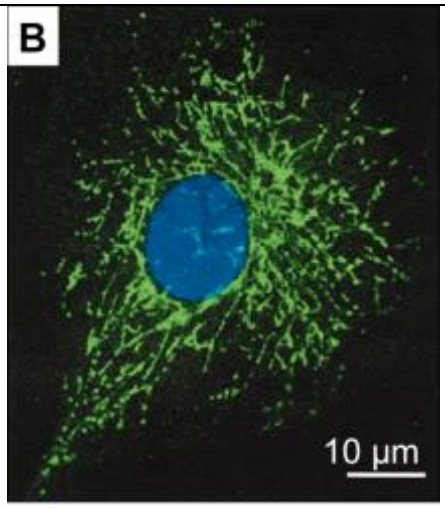
Both of the micrographs below show mitochondria.

- In the cells in (A), the mitochondria appear to each exist as a discrete unit.
- In (B) the mitochondria are green and appear to form a network in the cell (the nucleus is stained blue).

a) Which is a more accurate view of the **distribution** of mitochondria in a cell? Why?

b) What type of question would be best answered by the image that you did not pick in a)?





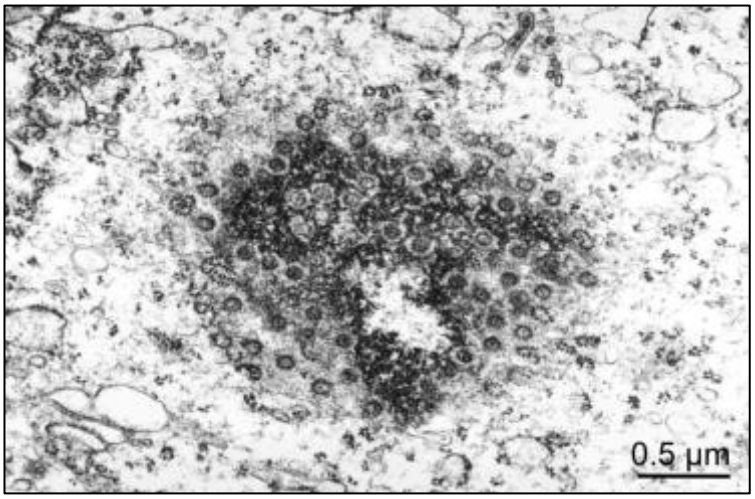
Problem 1.2.5 (Covered in Tutorial)

(tags: #TEM, #nucleus, #plane of section)

This transmission electron micrograph is of the nuclear envelope (dark material in center of image) and shows many nuclear pores.

a) Draw a circle on your paper to represent a nucleus. Next, draw a line that shows the plane of section that produced this micrograph.

b) Now write out a brief description of what you were illustrating in a). Show it to a classmate and see how well it describes what you're trying to explain (this is to help you practice writing out good answers for the exam).



Problem 1.2.6 (Covered in Tutorial)

(tags: #identify microscopy type)

In each of the experimental setups described below, choose the type of microscopy you would use to perform the experiment. If there is more than one way to visualize that would work, consider the pros and cons of each possibility and pick the best one. Be prepared to justify your answer.

a) Seeing a virus as it crosses the nuclear pore complex.
b) Localizing a green fluorescent protein (GFP)-tagged protein in the cytosol.
c) Visualizing the surface of a fungal cell.
d) Tracking mobile cancer cells in cell culture.
e) Viewing mitochondrial ribosomes.
f) Counting the number of mitotic chromosomes extracted from a cell.
g) Examining the arrangement of waxes on the surface of an epidermal leaf cell.
h) Visualizing transport of vesicles from the Golgi apparatus to the plasma membrane.
i) Looking at the arrangement of microtubules in an entire cell.
j) Examining the location of a specific resident protein inside an individual cisternae of the Golgi apparatus.

Problem 1.2.7 (Covered in Tutorial)

(tags: #identify microscopy type, #organelle identification, #plants, #animals)

For the images below, name the type of microscopy used and identify the organelle indicated by the arrow.

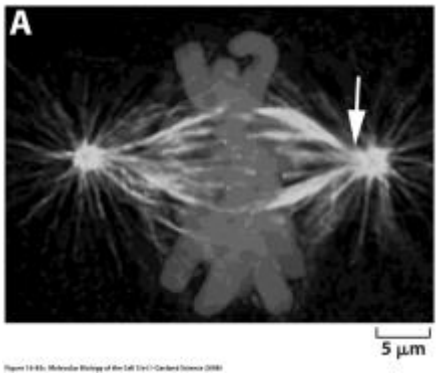


Image A
Type of Microscopy: _____
Organelle: _____

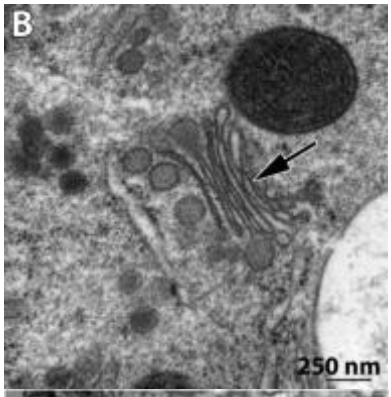


Image B
Type of Microscopy: _____
Organelle: _____

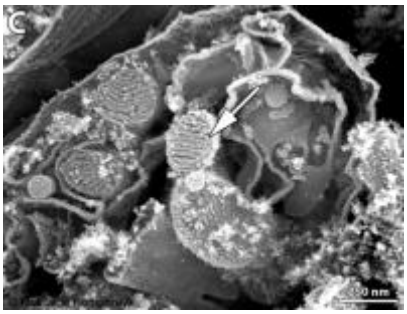


Image C-
Type of Microscopy: _____
Organelle: _____

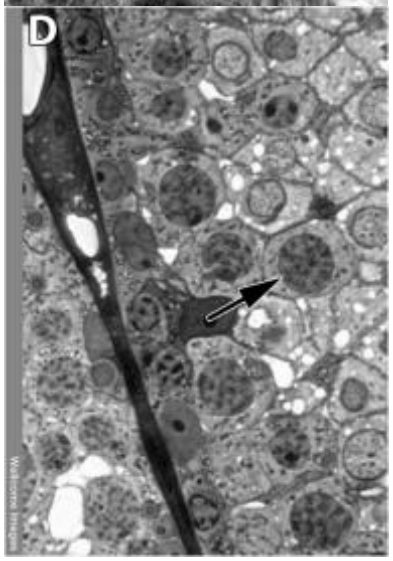


Image D-
Type of Microscopy: _____
Organelle: _____

Unit 2: Biological Membranes
Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 2.1 – Review of the Features of Biological Membranes

Lipid Questions

a) Find images of the following molecules in your textbook or online (2).
   a. 2 different phospholipids of your choice. Take note of their names. What do their names tell you about them?
   b. Cholesterol (for an extra challenge find an equivalent plant sterol)
   c. A glycolipid of your choice (For an extra challenge find 2)
   d. Sodium dodecyl sulfate (SDS, a detergent)

   Examine the molecular structures and label the following regions (note that they may not all exist in all of the molecules):
   - The polar region (differentiate between charged and uncharged molecules of the polar region) and the non-polar region
   - A region that would be stiff and inflexible
   - A glycerol residue (some molecules have a serine residue instead, does yours?)
   - A region that could easily have C=C bonds added (how would that effect the structure of that region?)

b) For the molecules you drew above, identify which ones would be able to form lipid bilayers on their own. Use the details of the structures that you drew to explain why or why not. (2)

Protein Questions

a) Define the 4 levels of protein folding, and explain how each one is stabilized (1).
b) Self-assembly of macromolecules is an important concept. What do you think that means? What is its relation to denaturation and renaturation? (2)
c) What about disulfide bridges? Are they covalent or non-covalent interactions? How do they form? Why are they considered to be uncommon in the cytosol? (2).

d) Find images of all 20 amino acids (don't memorize them!) online or in your textbook. Critically assess the structures, and identify the following (3):
   - The portion of the amino acid that is common to all of them.
   - The portion that is unique to each amino acid, known as the R-group or side-chain.
   - The functional groups that will form the peptide bond. Will anything be lost/ gained during that reaction? How do these parts relate to the 'N' and 'C' terminus of a protein?
   - The next questions are specifically for the R-groups. Based solely on their structure, identify the following:
     - Any R-groups that you would expect to have ACIDIC properties. Will they gain or lose a proton during that reaction?
     - Any R-groups that you would expect to have BASIC properties. Will they gain or lose a proton during that reaction?
     - Any R-groups that would not be able to form H-bonds with water
     - R-groups that would form H-bonds, but would not be acidic or basic.
     - R-groups that could interact ionically with their neighbours?
     - Any R-groups you would consider to be 'big' or 'small' relative to the others?

e) Compare what you've learned during this structural analysis of the R-groups to how they are categorized by your textbook, and try and justify those categories to yourself. Make sure you know the 1- and 3-letter codes for each amino acid (2).

Topic 2.2 – The Lipid Bilayer

f) What are the major differences between a synthetic phospholipid bilayer and a biological membrane? (2)
g) How do cells adjust maintain fluidity of their lipid bilayers in varying conditions? (1)
h) Despite appearances, cholesterol cannot form bilayers on its own. Use the structure of the molecule to explain why. (3)
i) Explain how the structure of phospholipids is the basis of the major properties of the bilayers that they form: physical form of the bilayer, self-sealing property, selective permeability, and fluidity of the bilayer. (3)
j) Why are lateral movements of phospholipids in a bilayer so much easier than 'flips' from one leaflet to the other? Explain why this means that we sometimes call a biological membrane a 'two-dimensional fluid' (2)

Topic 2.3 – Membrane Proteins

a) What is the difference between integral and peripheral membrane proteins? Discuss and compare the different strategies for association of proteins with membranes. (1)
b) What is a domain in a protein? How does it relate to structure and/ or function? (2)
c) Compare the hydrophobic forces that hold a membrane protein in the lipid bilayer to those that help proteins fold into a unique three-dimensional structure? (2)
d) How do cells restrict the movement of membrane proteins? Outline the different strategies and provide brief examples. (2)
e) How does membrane structure allow them to have separate identities and functions on each side? (2)

Practice Problems

- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- 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 2.1 – Review of the Features of Biological Membranes

Problem 2.1.1

(tags: #membrane permeability, #plasma membrane, #eukaryotic cells, #animals)

Organism	**Environment**	**Permeability of membrane for water** (um^3 of water/um^2 of surface/atm of difference in osmotic pressure between inside and outside of the membrane/ min)
Amoebae (Amoeba and Pelomyxa)	Fresh Water	0.023
Ciliates (Vorticella and Paramecium)	Fresh Water	0.25
Echinoderm egg (Arbacia)	Marine	0.4
Human Red blood cell	Serum	3.0

Data from Giese, Cell Physiology 1st ed. 1957

a) Would you expect the permeability of a phospholipid bilayer to be greater or less than that of any of these real membranes? Why?

b) What do you think is the basis of the difference in permeability of these membranes?

Problem 2.1.2

(tags: #membrane fluidity)

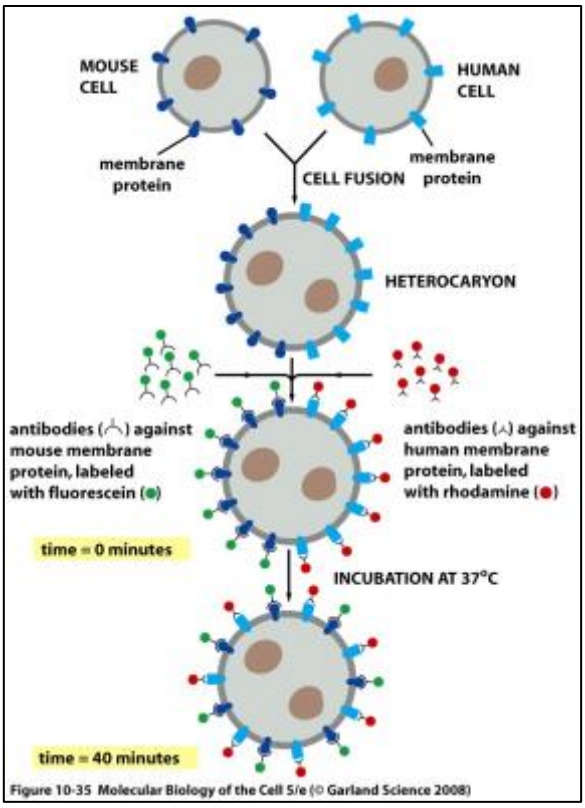
The figure on the right (similar to figure Fig 11-30; 3rd ed. 11-32) is an image of what is known as a hybrid cell experiment. Two cells that have different fluorescent labels on the plasma membrane are forced to fuse with each other (with the help of viruses).

If the resultant 'hybrid' cell is incubated at 37 degrees Celsius, the different fluorescent tags are seen to mix with each other.

a) Why would this happen? Explain the experimental results using information that you have learned in this unit.

b) What do you think would happen if the cells were cooled to a temperature below the critical temperature for the membrane after the cell fusion was completed?

c) What kind of controls might you need for this experiment?



Problem 2.1.3 (ONE of these Covered in Tutorial)

(tags: #essay outline, #membrane fluidity, #animals)

Formulating an outline for a good argument and supporting it with evidence is a difficult task, but one that we will expect you to be able to do over and over again in this course. It is also a key skill required when engaging in scientific thought. In this problem we are giving you some practice questions to try out writing outlines for. Remember to include a thesis statement and 3 arguments, each with supporting evidence. Also remember to write in outline format and clearly identify each part in your answer. Check the index for more questions like this one throughout the problem sets.

a) Phospholipids play an essential role in the formation and maintenance of the plasma membrane. Describe the structure of the plasma membrane (include all components) and explain how the properties of phospholipids, make them ideal molecules to be involved in membrane formation and maintenance.

b) Membrane fluidity varies widely across different eukaryotic organisms, based on the conditions of their environment. For example, frogs survive the winter in sub-zero temperatures without freezing into an ice cube. For this reason, these cold-blooded amphibians have always been of great interest to scientists. Discuss the factors that influence membrane fluidity and describe how cold-blooded animals (like frogs) could use them to survive the cold winters without freezing.

c) Phospholipids are capable of moving laterally within a single leaflet of a biological membrane, but only move from one leaflet to another with difficulty. Explain why lateral movement within a single leaflet is more frequent than flipping between leaflets using the biochemical properties of lipids to support your arguments.

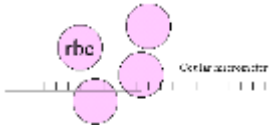
Problem 2.1.4 - Famous experiment - Gorter and Grendel 1927

(tags: #phospholipids, #plasma membrane, #animals)

Everything you've ever learned in Science at UBC is the result of someone somewhere asking a question, and then running an experiment to answer that question. As time has gone on the questions have gotten more complex, but the process is the same.

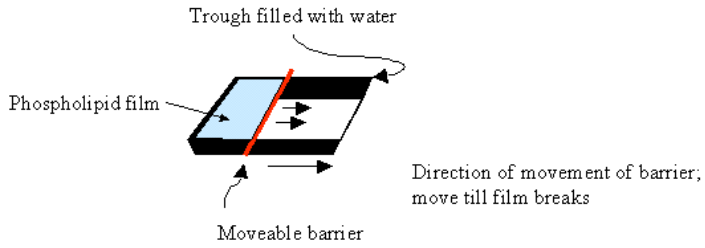
In 1927, Gorter and Grendel asked the question, "*How are phospholipids arranged in membranes?*"
Experimental Approach: Determine how much phospholipid is contained in a single unit area of plasma membrane from a red blood cell.
Procedure:

1. Determine number of washed cells per ml.
2. Measure the area of plasma membrane by putting cells on microscope slides and measuring the diameters with a micrometer. The surface area of the plasma membrane per cell was then estimated mathematically.



3. Extract phospholipids with ether and then concentrate them.
4. Spread a known fraction of the lipid material on the surface of water in a Langmuir-Blodgett trough and determine the total area of lipid 'slick' formed.

A Langmuir-Blodgett trough is a tray full of water to the brim with a moveable barrier across the middle. The phospholipid material dissolved in ether is added to one side, and the ether is allowed to evaporate. The phospholipid material forms a 'slick' behind the barrier. The barrier is then moved to the right to increase the area of the slick. Initially the phospholipid molecules will be piled up several deep. As the area of the slick is allowed to expand the slick will gradually form a molecular monolayer. Eventually the area behind the barrier will be greater than the area of the lipids as a monolayer and the slick will tear or fail to cover the whole area. The thickness of the phospholipid film can be judged by the color of reflected light so they could tell when it was all a uniform thickness.



Results: In several experiments, using red blood cells from different mammals, they obtained areas of phospholipid film that were between 1.8 and 2.1 times the calculated area of the membrane.

Based on this information: What would you conclude about the arrangement of phospholipids in a membrane?

Problem 2.1.5 (Walkthrough Available)

(tags: #transmembrane domains, #protein structure, #FRAP, #animals)

a) The transmembrane protein glycophorin spans the lipid bilayer of a red blood cell with a single α-helix. Which of the 3 amino acid sequences below is the most likely to be the membrane-spanning domain of this protein? Explain your reasoning.

I T **L** I Y F G **V** M A G V I G T **I** L L I S
I T **T** I Y F G **S** M A G V I G T **Y** L L I S
I T **E** I Y F G **R** M A G V I G T **D** L L I S

b) You want to run an experiment to see whether glycophorin is anchored to another protein inside the cell. You decide that a FRAP experiment is the best way to address this question. Why?

c) Your results indicate that Glycophorin is, in fact, anchored. Draw and label the graph of your results from the FRAP experiment for Glycophorin. If glycophorin were not anchored, how would the graph you drew be different?

Problem 2.1.6

(tags: #mutant analysis, #protein sequence, #animals)

Here are partial amino acid sequences of normal human hemoglobin and three variants (mutants) that occur normally in the human population. Some of these hemoglobin molecules lead to defective function of the molecules while others function quite normally.

Type of Hemoglobin (Hb)	Amino acid number								
	3	4	5	6	..9..40		41	42	43
Normal beta Hb	Leu	Thr	Pro	Glu	Ser	Gln	Arg	Phe	Glu
Mutant Allele 1	Leu	Thr	Pro	Val	Ser	Gln	Arg	Phe	Glu
Mutant Allele 2	Leu	Thr	Pro	Glu	Ser	Gln	Arg	Ser	Glu
Mutant Allele 3	Leu	Thr	Pro	Glu	Thr	Gln	Arg	Phe	Glu

a) Which alleles are most likely to produce abnormal phenotypes? Why?

b) Which are least likely to produce abnormal phenotype? Why?

c) Mutant Allele 1 is also the one that causes sickle cell anemia. The mutated form of hemoglobin is the same shape as the normal type, but the hemoglobin proteins clump together, forming large deposits in red blood cells that affect both structure and function of the entire cell. Explain how this particular amino acid substitution could cause clumping of hemoglobin without affecting the overall shape of the protein.

Problem 2.1.7 - The Coast Salish Wool Dog

(tags: #protein sequence, #protein structure, #prediction, #animals)

"Coast Salish" refers to a group of First Nations peoples that live in the Pacific Northwest and Includes the Musqueam peoples whose traditional territories encompass UBC's Vancouver campus. The coast Salish peoples were reported to have used hair from a specific species of small white dog, unique to this area of Coastal BC, in their woven blankets. These dogs, along with many other species of North American native dogs, are thought to have been domesticated separately from their European counterparts before the arrival of Europeans in North America.

(Image Source: Stockford & Pye (1998). Cdn J of Archeology 21: 149-153)

(Image source: "Woman Weaving a Blanket", Paul Kane, Royal Ontario Museum)

Unfortunately, the arrival of Europeans (in the 1800s) marked the beginning of the extinction of these wool dogs, as sheep wool (and Hudson's Bay wool blankets) became more widely available. The wool dog slowly lost its distinct identity, as interbreeding continued, and the last known identifiable wool dog died in 1940. Goat hair is also used in native weaving, more commonly found in ceremonial blankets. Much archeological research is done on the domestic animals of North American native peoples and how they were used in everyday life.

Mammalian hair is made of an intermediate filament protein known as keratin. Intermediate filaments are multi-subunit complexes made entirely of one type of subunit (in this case its keratin). We will speak more about intermediate filaments in Unit 7. This hair is evolutionarily conserved between species, thus the primary sequence of the keratin from each species is slightly different. Scientists can remove small amounts of hair from the fiber in the blankets remaining in the archeological record, and analyze them to compare the primary sequences between species. Some of that data is below (Solazzo et al (2011) *Antiquity*, 85: 1418-32).

Species	**Partial primary sequence of keratin protein**
Dog (*Canis lupus familiaris*)	SDLEAQVESLREELLSLK
Mountain goat (*Capra hircus*)	SDLEAQVESLKEELICLK
Domestic Sheep (*Ovis aries*)	SDLEAQVESLKEELICLK

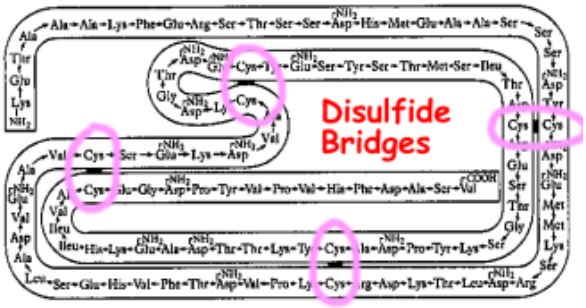
a) Are there any differences in the primary sequence of keratin from these 3 species? If yes, what are they?

b) Would you predict that these differences in primary structure would result in a difference in the quaternary structure of the intermediate filament? Why or why not?

Problem 2.1.8 (Walkthrough Available)

(tags: #protein structure, #animals)

The experiment described here is of historical importance as it provided the first decisive evidence that the folding of proteins was dependent only on the primary structure of the protein and that it was a result of molecular self-assembly (i.e. based on chemical properties). A Nobel Prize was awarded for this work in 1972.

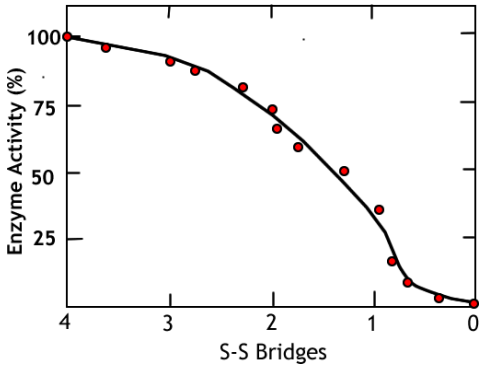


Background: This experiment deals with the structure and activity of ribonuclease, an enzyme that degrades RNA. It is produced in the pancreas. It is one of the first proteins for which the amino acid sequence was known. The protein's enzyme activity occurs only when the molecule is properly folded. As the molecule is denatured, the enzymatic activity is lost.

The molecule has 4 disulfide bonds (C-**S-S**-H) that hold the chain together (see picture on the right). These form between cysteine residues. If the molecule is to be completely denatured, these bonds must be broken. Disulfide bridges are broken by placing the protein in a solution of 8 M urea and beta mercaptoethanol (HS-CH_2-CH_2-OH, reducing agent). The urea interferes with formation of hydrogen bonds and the reducing agent provides a reducing environment, in which the disulfide bond will break, leaving 2 –SH groups (one on each cysteine).

The plot on the right (Figure 1, top) shows how the enzymatic activity decreases as the mean number of disulfide bridges per ribonuclease molecule decreases during denaturation. Each disulfide bridge produces two SH groups:

- C- S- S- C- → C- SH HS- C-
(oxidized) (reduced)



If the reducing agent and the urea are removed the protein will slowly oxidize in the presence of air. The S-S bonds will reform and enzyme activity is restored, as shown in the lower graph on the right (Figure 2).

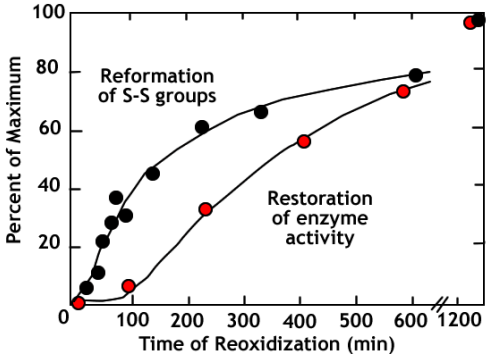
a) Describe how enzyme activity changes as the mean number of disulfide bridges decreases. Why do you think the enzyme activity is affected this way?

b) Compare the changes in enzyme activity and reformation of S-S groups change as reoxidization progresses. What does this tell you?

c) What are some of the assumptions we are making in this experiment?

d) Why do you think there is a lag between the formation of S-S bonds and the restoration of enzyme activity?

e) Based on the data been presented here, what did this landmark research contribute to our understanding of protein folding?



(Source of data: Anfinsen et al. 1961 PNAS 47, 1309, and White 1960 J. Biol. Chemistry 235, 383)

Topic 2.2 – The Lipid Bilayer

Problem 2.2.1

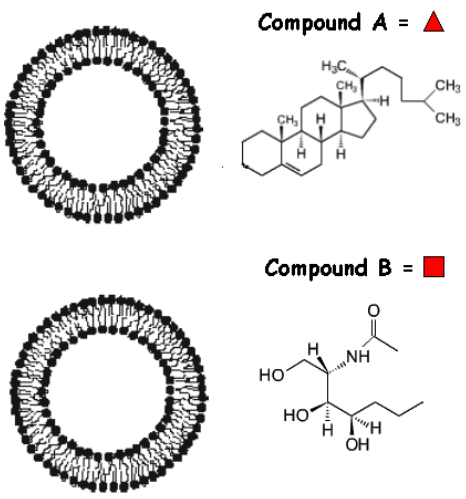
(tags: #hydrophobicity)

You are the president of a local biotech company that develops liposome-based pharmaceuticals.

Liposomes are vesicles surrounded by a phospholipid bilayer. They can be prepared with solutions containing drugs on the inside, and can deliver these compounds to target cells through fusion with the plasma membrane, releasing their contents into the cell

A researcher at UBC has discovered two novel compounds that have anti-tumour activity. She would like you to insert the drugs (known as Compound A and B) into 2 separate liposomes, in order to facilitate delivery to human cells.

Assume that you have the expertise required to encapsulate the drugs (get them into the liposome). Based on the structure of each compound, draw where in the liposome each drug would be localized in the final product. Provide a brief statement of rationale for your choice. For simplicity, use a triangle or square to represent each drug.



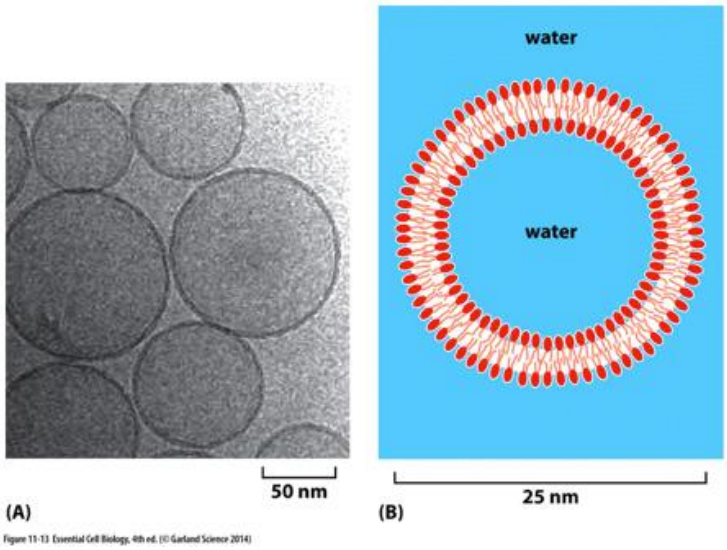
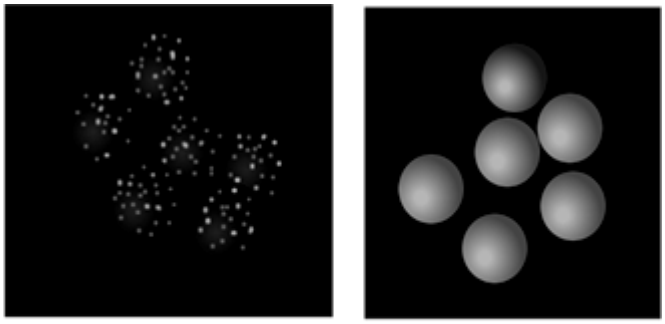


Figure of Liposomes: (A) TEM of phospholipid vesicles (liposomes) showing the bilayer structure of the membrane. (B). A drawing of a small spherical liposome seen in cross section.

Problem 2.2.2

(tags: #fluorescence microscopy, #plasma membrane)

An experiment was run to examine the distribution of phosphatidylserine in the plasma membrane of red blood cells. Membranes were isolated, broken into large fragments, and then left to reform into vesicle-like structures known as microsomes. From this, two separate populations of microsomes have been identified and are present in a buffer solution:



Right-side-out vesicles (ROVs) | Inside-out vesicles (IOVs)

(1) right-side-out microsomes, in which the extracellular leaflet of the vesicle faces the buffer (equivalent in orientation of the intact plasma membrane); and

(2) Inside-out microsomes, in which the extracellular leaflet faces the interior of the vesicles and the cytoplasmic leaflet faces the buffer.

Annexin V is a protein that binds to phosphatidylserine, one of the phospholipids present in cell membranes. Fluorescently labeled annexin V is added to the buffer containing each of the membrane preparations (ROVs and IOVs), and incubated briefly to allow labelled annexin V to bind to its target. The microsomes are then washed to remove unbound annexin V and finally examined by fluorescence microscopy. Presence of annexin V is detected by bright fluorescence against a dark background, as shown above.

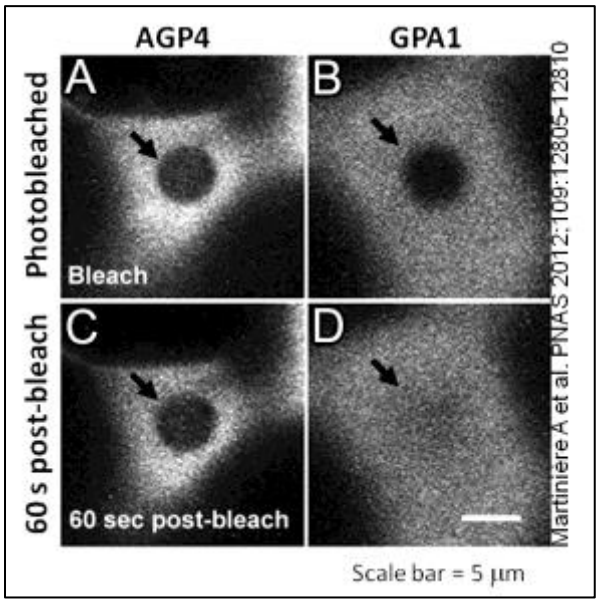
a) Describe what can be observed in each panel.

b) What conclusions can you draw about the distribution of phosphatidylserine in the plasma membrane of the red blood cell? Explain your reasoning.

Problem 2.2.3

(tags: #FRAP, #fluorescence microscopy, #membrane fluidity, #plants)

Martiniere et. al. (2012) tagged two plant membrane proteins (AGP4 and GPA1) with GFP so that they could be detected in cell membranes. After tagging, they carried out FRAP experiments to examine the mobility of AGP4 and GPA1. The photobleached region of the cell membrane is indicated by an arrow.



a) Describe what you observe in each panel (A – D).

b) When you compare all panels together, what can you conclude about AGP4 and GPA1 mobility?

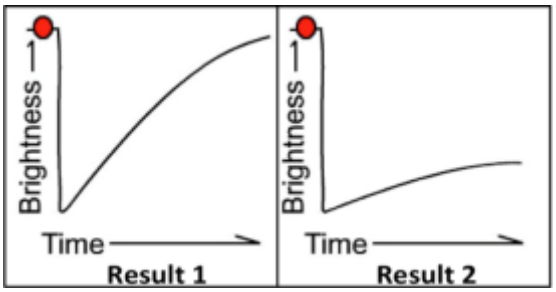
c) What could account for the differences in mobility between AGP4 and GPA1 that you observed?

d) Draw the FRAP curves that you would expect to see for these two membrane proteins.

Problem 2.2.4

(tags: #FRAP, #membrane fluidity, #prediction)

A FRAP experiment was done to compare the movement of a specific membrane protein in the plasma membrane in 2 different cell types. The protein was fluorescently-tagged using GFP, and then followed using FRAP. Here are the results. Result 1 is from Cell Type 1 and Result 2 is from Cell Type 2.



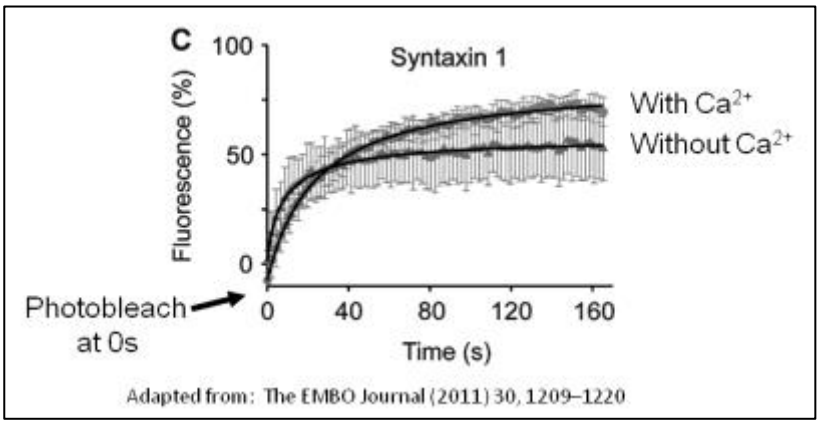
a) What can you conclude about the fluidity of the membrane in Result 2 compared to Result 1?

b) Using the list of factors below that effect membrane fluidity, predict what could be contributing to the different results?

- Saturation of lipids
- Length of fatty acid tails
- Amount of sterol in the membrane
- Temperature of the environment
- Connections with the cell cortex

Problem 2.2.5

(tags: #FRAP, #membrane fluidity, #animals)



Calcium ions (Ca^{2+}) are often used by the cell to modify the behaviour of other molecules. Here, Zilly et al. (2011) conducted an experiment to look at the effect of Ca^{2+} on the mobility of the transmembrane protein Syntaxin 1. Fluorescently labelled Syntaxin 1 was expressed in cells and FRAP experiments were carried out in the presence or absence of Ca^{2+}.

a) Describe the change in fluorescence recovery of cells with and without calcium ions.

b) Which treatment is experimental control? Explain.

c) Based on the results, what can you conclude about the effect of Ca^{2+} on the mobility of Syntaxin 1?

Topic 2.3 – Membrane Proteins

Problem 2.3.1

(tags: #protein structure)

Two proteins, A and B, are the same size. Each is a single polypeptide chain with 200 hydrophilic amino acids and 100 hydrophobic amino acids. Protein A is an integral membrane protein, and Protein B is soluble in water.

a) For each protein, explain what you would expect to observe at each level of protein folding (1°, 2°, 3°, 4°)

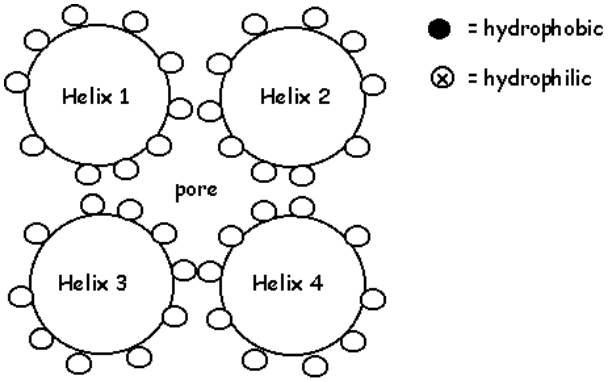
b) Why does the environment that the protein is in matter?

c) What would happen to Protein B if you put it in oil instead of water? What about if you put protein A in water instead of the membrane?

Problem 2.3.2

(tags: #transmembrane domains, #protein structure)

The diagram on the right shows a top down view of a membrane pore. An alpha helix has 3.7 amino acid residues per gyre (=rotation). The side chains extend outwards from the helix.



Based on your knowledge of amino acid and membrane chemistry, place the amino acids in the proper locations on the helices in order to build a pore that could be used to pass water through a biological membrane.

Problem 2.3.3

(tags: #transmembrane domains, #protein structure, #protein sequence, #prediction)

a) Make a prediction about the primary sequence you would expect to find in a membrane-spanning β-barrel. Which of the following sequences best match with your prediction? Explain.

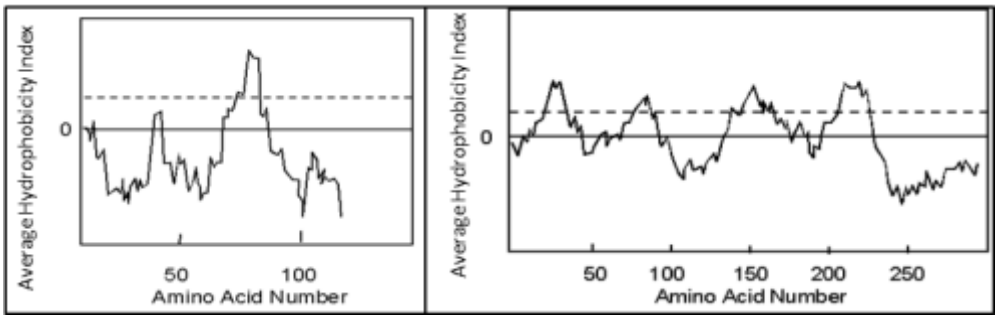
1. A D F K L S V E L T
2. A F L V L D K S E T
3. A F D K L V S E L T

b) Why do membrane-spanning regions almost always have ordered secondary structure, instead of being a random coil?

Problem 2.3.4

(tags: #transmembrane domains, #hydrophobicity plots, #prediction)

The 2 graphs below each show a hydrophobicity plot for a trans-membrane protein. Hydrophobicity plots are a bioinformatic tool that help predict whether a given protein has a hydrophobic region big enough to cross a membrane. It is based on a calculation of the free energy (ΔG) required to immerse each individual amino acid in water. The data are plotted as a running average of three consecutive amino acids, starting at the N terminal end.



a) Would you predict that either of these proteins have a membrane-crossing domain? Explain your reasoning.

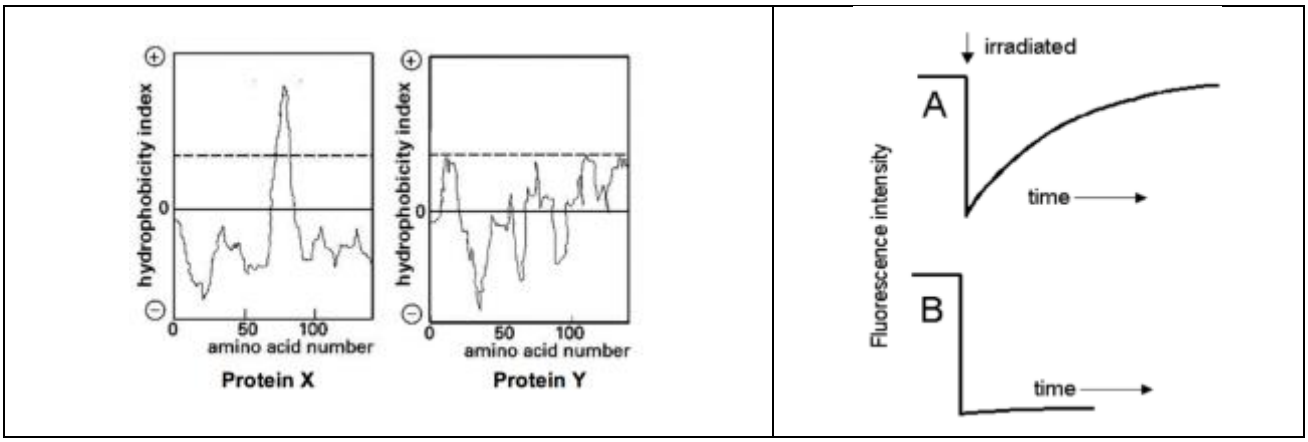
b) If you used this tool to try and predict a membrane spanning region for your protein in the previous problem, it would be unsuccessful, despite the fact that 4 membrane-spanning domains exist. Why is that? What does that tell you about predictive bioinformatics tools in general?

Problem 2.3.5

(tags: #FRAP, #hydrophobicity plots, #membrane fluidity)

a) The graphs on the left show two hydrophobicity plots for 2 membrane proteins. Which of these proteins is more likely to have a membrane crossing domain?

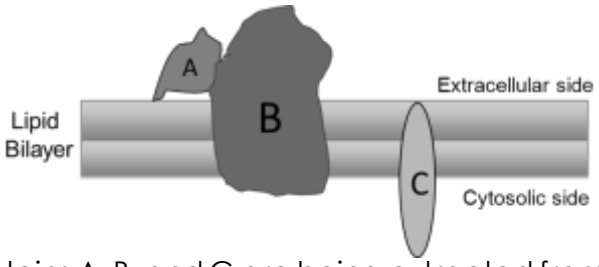
b) To analyze the mobility of these membrane proteins in membranes of similar composition, you performed a fluorescence recovery after photobleaching (FRAP) experiment. The figure below shows your results. Note that Graph A corresponds to Protein Y, and Graph B corresponds to Protein X. What do the FRAP results tell you about the two proteins? Explain.



Problem 2.3.6 (Walkthrough Available)

(tags: #SDS-PAGE, #protein topology, #prediction)

Shown below is a drawing representing the arrangement of proteins on plasma membrane of a cell.



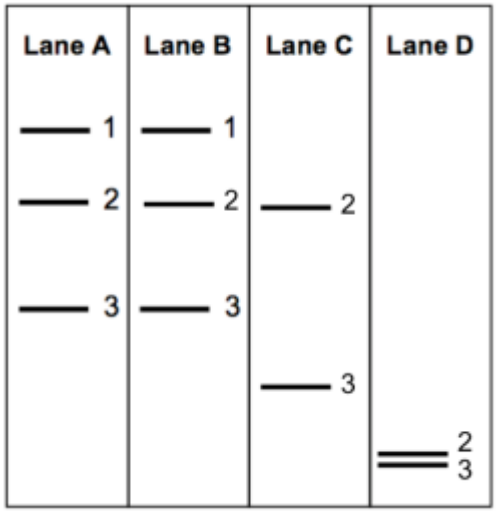
In the following experiment, proteins A, B, and C are being extracted from membranes (pictured above) after various experimental treatments (listed below). The extracted proteins will then be separated by size using gel electrophoresis (SDS-PAGE). Predict the results that you would expect to see in this experiment by drawing clearly labeled protein bands on the gel image below.

Lane 1: intact cell with **no** protease treatment
Lane 2: Brief detergent treatment first with **no** protease treatment
Lane 3: membrane solubilized completely by detergent first followed by protease treatment
Lane 4: intact cell + treatment with a solution of high salt concentration + **no** protease treatment
Lane 5: intact cells + protease treatment
Lane 6: Brief detergent treatment first + protease treatment

Problem 2.3.7

(tags: #SDS-PAGE, #protein topology)

Shown here are drawings representing the results of another SDS-PAGE analysis of three membrane proteins treated in a variety of ways. The treatments used were:



Lane A: total membrane proteins from intact cell with **no** trypsin treatment.
Lane B: total membrane proteins from partially digested membrane first with **no** trypsin treatment.
Lane C: proteins from intact membrane treated with trypsin, an enzyme that digests proteins.
Lane D: proteins from partially digested membrane treated with trypsin.

Based on this data, draw a lipid bilayer with each of these membrane proteins in its proper place. Write a short paragraph explaining your logic.

Problem 2.3.8 (Covered in Tutorial)

(tags: #SDS-PAGE, #protein topology)

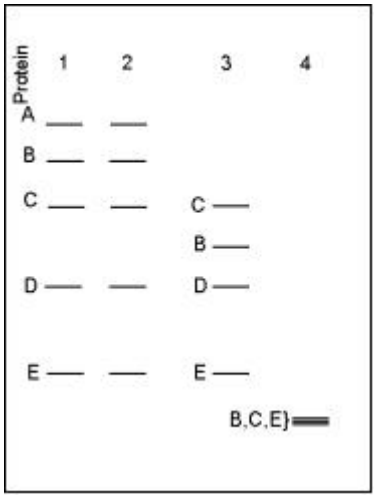
SDS-PAGE is often used to determine the location and orientation of membrane associated proteins. Commonly, experimental preparations of membranes are treated in a variety of ways in order to extract the proteins, which are then subjected to SDS-PAGE. These treatments will affect membrane proteins in different ways, based on how they are arranged in the membrane.

One of the most common pre-treatments is by the digestive enzyme trypsin, which degrades polypeptide chains. Trypsin is too large to cross a membrane, but can digest exposed parts of membrane proteins. Those parts of a membrane protein that are in contact with external aqueous medium containing trypsin are digested, but the enzyme cannot digest the parts of the protein that are embedded within the lipid bilayer, or the parts of the proteins on the cytoplasmic side of the membrane if the cell is intact. If detergents are used to gently permeabilize the membrane preps prior to placing them in trypsin containing medium, the enzyme can access both sides of the membrane and will digest any polypeptide chains that is exposed and accessible.

a) Draw a portion of a plasma membrane of a cell and label the cytoplasmic and extracellular surface. Place the following four proteins in the membrane: a) peripheral membrane protein (cytoplasmic exposure), b) integral protein (transmembrane exposure), c) integral protein (extracellular exposure) and d) peripheral protein (extracellular exposure)
b) Describe what will happen to each of your proteins when this cell is exposed to trypsin only.
c) Describe what will happen to each of your proteins when this cell is treated briefly with a detergent first, and then exposed to trypsin.

Experimental Design and Procedure: In this experiment, the proteins were extracted from the membranes with detergent and separated by SDS-PAGE. Prior to this, the cells were subjected to the following four treatments:

- *Treatment 1:* Intact cells, no trypsin treatment.
- *Treatment 2:* Brief detergent treatment first, no trypsin treatment.
- *Treatment 3:* Trypsin treatment of intact cells.
- *Treatment 4:* Brief detergent treatment first, followed by trypsin treatment.



Membranes contain many different types of proteins. In this experiment, five different proteins were identified using antibody labeling. Each antibody will recognize its target protein, but not other proteins. The results can be seen in the image on the right.

d) Use the data for the figure above to fill out the table below.

Type	Location	Protein ID
Integral	Cytoplasmic exposure	
	External exposure	
	Transmembrane	
Peripheral	Cytoplasmic exposure	
	External exposure	

Unit 3: Nuclear Structure and Function

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 3.1 – Nuclear Structure & Protein Import

a) Discuss the relationship between the nuclear envelope and the endoplasmic reticulum. (1)

b) What is the functional role of the nuclear lamina? (1)

c) One of the key regulatory proteins that initiates the cell division process phosphorylates the proteins of the nuclear lamina. When this happens the proteins dissociate. From what you already know of the events of mitosis (from 1st year) what does this tell you about the role of the nuclear lamina? (3)

d) Explain how proteins are targeted to the nucleus. Specifically, explain the roles of the internal targeting sequence encoded in the protein, the specific receptor that recognizes these targeting sequences and the process by which the complex of nuclear destined protein and its receptor interact with components of the nuclear pore to enter the nucleus. (2)

e) Describe the three major structural elements of the nucleolus. (1)

f) Sketch a nucleus on your paper. Draw and label the...

- Nucleolus
- Nuclear envelope
- Nuclear pores
- Nuclear lamina
- Heterochromatin
- Euchromatin

Topic 3.2 – Chromatin and Chromosomes

a) Find structures of the 5 major nucleic acids in your textbook or online. Identify the following features on the structures (2):

- The ribose sugar. What is the major difference between this sugar in DNA & RNA?
- The phosphate group(s). Identify the bond broken when a nucleotide tri-phosphate is incorporated into DNA or RNA?
- The purine or pyrimidine ring. How do these structures contribute to base stacking and 3D structure of the final molecule?
- The H-bonds that are involved in base pairing.
- The 3' and the 5' ends of a strand of nucleic acids.

b) Use this structure as the basis for an argument to explain why base pairing is so precise. (2)

c) RNA is single stranded, but the bases are still capable of base pairing. Explain why this is an advantage for the cell. (2)

d) DNA and RNA are said to be polar because of the presence of the 3' and 5' end specifically, rather than due to any charges that might be present. Explain why this is so. (2)

e) Explain how proteins and DNA interact to form chromosomes, starting with the 2nm naked DNA molecule. (2)

f) Explain how the properties of the R-groups of the amino acids in histones must be arranged in order for them to be able to form the nucleosome core (i.e. they must be able to interact specifically with each other) and for them to promote interactions with the acidic backbone of the DNA (3).

Topic 3.3 – Regulation of Gene Expression

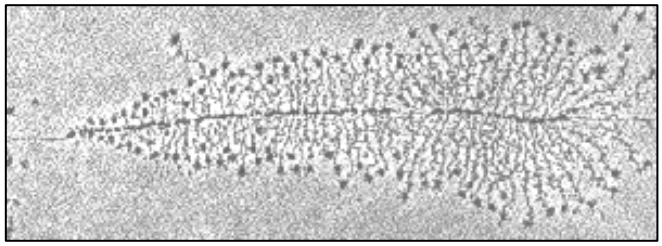
a) Differentiate between euchromatin and heterochromatin. (1)

b) Facultative heterochromatin consists of the same DNA sequences that are present in euchromatin. Yet, the euchromatin sequences are transcriptionally active and the heterochromatic sequences are inactive? What could be happening to account for the difference? (3)

c) Where and how are histones most commonly modified? What is the result? (2)

d) What are chromosomal loops? (1) What is their functional significance? (2) Compare and contrast eukaryotic and prokaryotic transcription. (1)

e) What is going on with the eukaryotic transcription unit in the image shown? Where is the DNA? Where are the polymerases? What is the orientation of the copied DNA strand (5', 3')? Where are the 3' and 5' ends of the transcripts? (2)



f) What are transcription factors? How do they bind to DNA? (1)

g) What's the difference between a basal transcription factor, an enhancer & a suppressor? (1)

h) Define the following: 5' cap, intron, exon, spliceosome, snRNPs, polyadenylation site, alternate splicing, exon choice, and promoter choice. (1)

i) snRNPs play a critical role in the intron excision process. What is this and why is it important that the process of intron excision be absolutely precise? (2)

j) Alternative splicing patterns is usually tissue-specific. Promoter choice is also often region, stage or tissue specific. Explain how eukaryotes use these strategies to their advantage. (3)

k) What are the implications of universality of the code in different organismal kingdoms? How does modern science benefit from this concept? (3)

Practice Problems

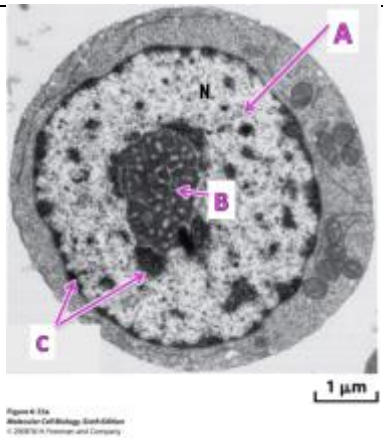
- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- Note that the problems are roughly divided by topic, but you should expect to use knowledge from other parts of the course as well.

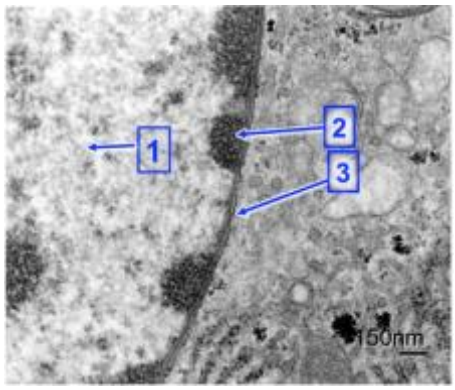
Topic 3.1 – Nuclear Structure & Protein Import

Problem 3.1.1

(tags: #nuclear structure, #TEM, #animals)

Identify the structures labeled on the micrographs and describe the role of each one.





Problem 3.1.2

(tags: #nuclear targeting, #cell cycle)

During cell division, nuclear proteins are dispersed into the cytoplasm when the nuclear envelope breaks down. In interphase, they are once more localized to the nucleus. Explain how the dispersed nuclear proteins can be returned to the nucleus after cell division.

Problem 3.1.3

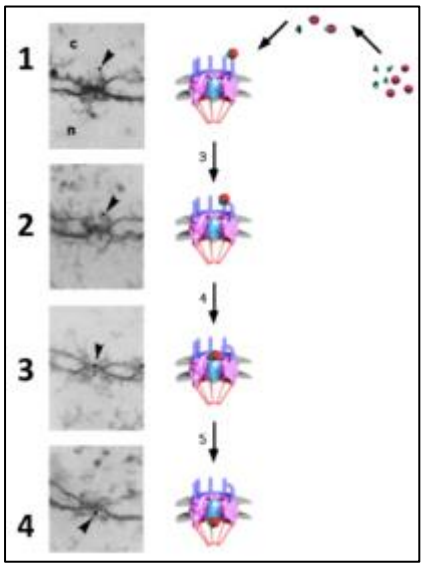
(tags: #nuclear structure)

You treat a cell with a strong anionic detergent you find in the lab. You find that although the detergent has successfully dissolved the nuclear envelope, the nucleus itself is still intact. What is responsible for maintaining the nuclear structure? Explain.

Problem 3.1.4

(tags: #nuclear targeting)

There are 2 primary mechanisms for nuclear transport, one of which is shown here.



a) Briefly explain the 2 ways that molecules can enter the nucleus.

b) Identify which type is shown here and describe the 4 stages of the process as imaged.

Problem 3.1.5

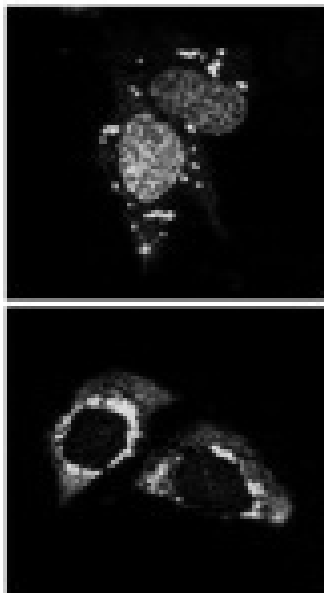
(tags: #fluorescence microscopy, #NLS, #experimental controls, #animals)

FI16 is a protein that detects the presence of viral DNA in the cytoplasm and nucleus of infected cells. The nuclear localization signal (NLS) of this protein contains a site that may be modified through acetylation– an acetyl group may be chemically attached. Here, Li et al. (2012) study the role of this acetylation site by looking at the location of GFP-tagged IFI16 proteins. What type of microscopy was used to image these cells?

GFP-tagged IFI16

Without acetylation

With acetylation



Adapted from Li et al. PNAS 2012;109:10558-10563

a) Describe in the fluorescent localization in each panel and how it changes.

b) Which panel would be considered to be a control in this experiment? What is the purpose of this control?

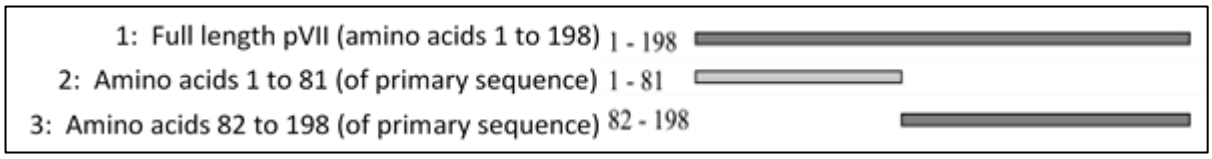
c) When you compare both panels together, what can you conclude about the role of acetylation in the function of IFI16? Explain.

Problem 3.1.6 (Walkthrough Available)

(tags: #fluorescence microscopy, #NLS, #mutant analysis, #animals)

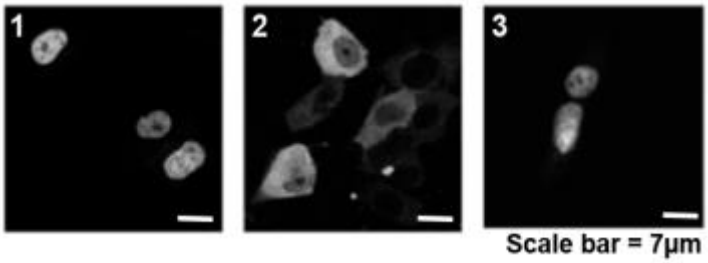
Wodrich et al. (2006) looked at the location of nuclear localization signals (NLS) within Adenovirus protein pVII. This is one of their experiments:

- Full length and deletion mutants of pVII were fused to GFP (green fluorescent protein) and injected into the cytoplasm of cells.
- Injected cells were viewed using fluorescence microscopy.



a) Identify the cellular location of the fluorescence in each panel (1, 2 & 3)

b) What can you conclude about the location of the NLS in pVII? Explain.

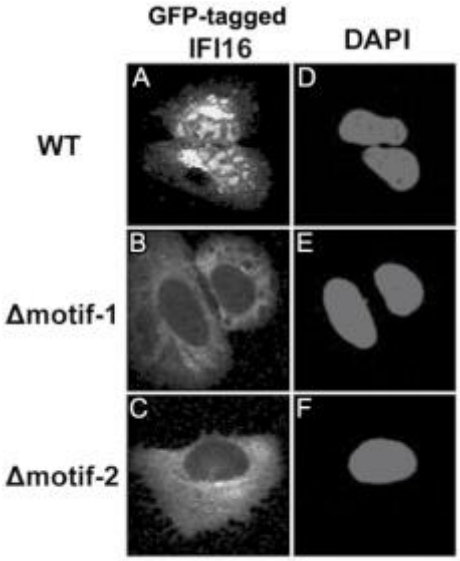


Problem 3.1.7

(tags: #identify microscopy type, #NLS, #mutant analysis, #animals)

IFI16 is a protein that detects the presence of viral DNA in the cytoplasm and nucleus of infected cells (Panel A). It has an unusual bipartite (i.e. two-part) nuclear localization signal (NLS).

In order to study the importance of both parts (motif-1 and motif-2) of the NLS in IFI16 targeting, GFP-tagged deletion mutants were made and expressed in cells. Δmotif-1 mutant is missing the first part of the NLS but still contains the second part, whereas Δmotif-2 mutant contains the first part of the NLS but is missing the second part. The same cells were also stained with DAPI.



Adapted from Li T et al. (2012) PNAS. 109: 10558-63

a) What type of microscopy was used to image these cells?

b) Describe the pattern of the fluorescence in panels A, B and C. What cellular compartments are being labelled in each panel?

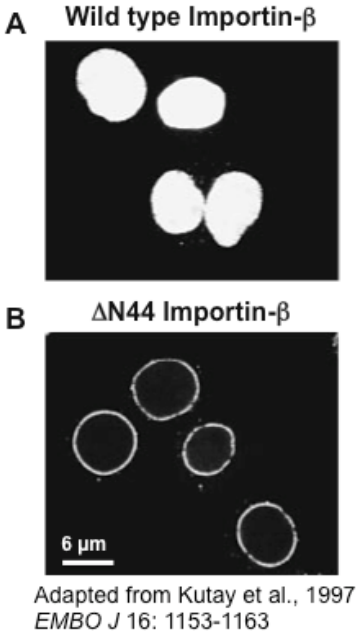
c) Why were the cells also stained with DAPI? What does this stain tell you?

d) When you compare all panels together, what can you conclude about the importance of motif-1 and motif-2 in IFI16?

Problem 3.1.8

(tags: #fluorescence microscopy, #nuclear targeting, #mutant analysis, , #plane of section, #experimental controls, #animals)

The protein Importin-β is a nuclear import receptor. To investigate the function of different parts of the amino acid sequence of Importin-β on the nuclear import of a nuclear protein called protein NP, researchers made a mutant form of Importin-β that lacks the first 44 amino acids. The mutated form of Importin-β is called ΔN44 Importin-β.



Adapted from Kutay et al., 1997
EMBO J 16: 1153-1163

They carried out an experiment using isolated nuclei, which were incubated with GFP-tagged NP, GTP and a GTPase (to provide cellular energy), and either wild type Importin-β or the mutant ΔN44 Importin-β. Their results are shown in the figures below. Note that these images were taken by fluorescence microscopy at the same plane of section through the nuclei. The magnification of both images is the same.

a) Which panel is showing the control? What is it controlling for?

b) Describe the pattern of the fluorescence in each panel. What compartment is being labelled in each panel?

c) What do these results tell you about NP nuclear import in each condition?

d) What conclusions can you draw from this experiment about the function of the first 44 amino acids of Importin-β? Justify your answer using the data provided.

Topic 3.2 – Chromatin and Chromosomes

Problem 3.2.1

(tags: #DNA packing, #protein structure, #essay outline #histones)

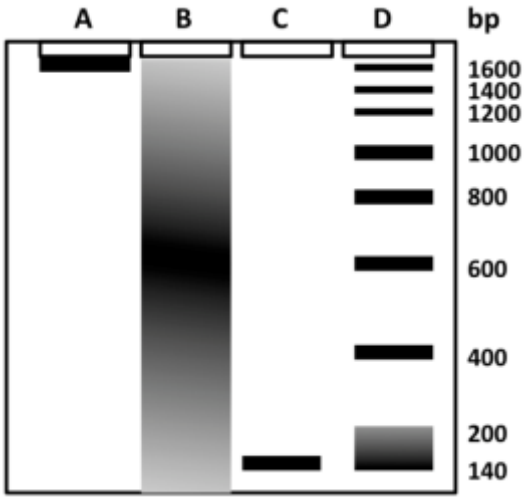
The ability to write strong supporting arguments takes practice. For the following statement, develop a thesis statement, a 3 supporting arguments, with evidence for each one. Remember to write in full sentences, but to keep it short and concise as well.

- Discuss the importance of the four levels of protein structure and noncovalent interactions between macromolecules in the packing of DNA into the eukaryotic nucleus.

Problem 3.2.2 (Covered in Tutorial)

(tags: #gel electrophoresis, #DNA packing, #nuclease digestion, #experimental controls)

Evidence for the packing of DNA in the eukaryotic nucleus originally came from experiments, such as the ones described in the next few problems. Researchers first postulated that nucleosomes existed in the eukaryotic chromatin, but then they had to prove it experimentally. One of the most powerful experiments that helped to prove the presence of nucleosomes was the nuclease digestion experiment, in which they used micrococcal nuclease to digest DNA. This enzyme is a large bacterial enzyme that can only cut exposed DNA (i.e. 'naked' DNA). It **cannot** cut DNA that is tightly bound to proteins.



Experimental Protocol:

0. Starting point: 30nm chromatin fibre.
1. Wash chromatin with weak salt solution to remove H1 histone.
2. Digest 11nm fiber with micrococcal nuclease (according to treatments listed below)
3. Remove associated proteins with a strong salt solution.
4. Separate DNA fragments using gel electrophoresis

4 different treatments are used in Step 2 (Treatment A = Lane A):

- **Treatment A:** No nuclease treatment.
- **Treatment B:** Brief (30sec) nuclease digestion of naked DNA.
- **Treatment C:** Extended (20min) nuclease digestion of chromatin
- **Treatment D:** Brief (30sec) nuclease digestion of chromatin.

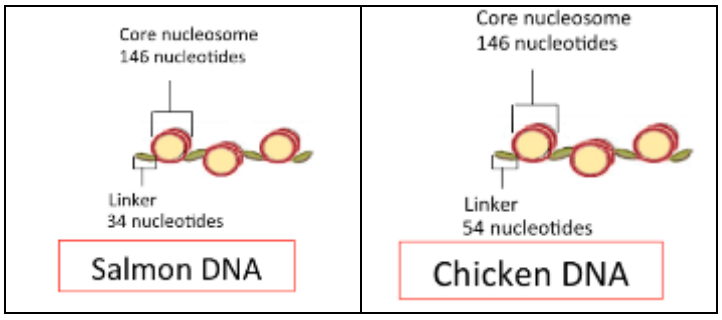
Questions:

a) Which treatments are the controls? What do they tell you?
b) What proteins remain associated with the chromatin after step 1?
c) How will the length of the nuclease digestion impact the results? Why?
d) For each step of the experiment, draw what the chromatin should look like.
e) Is there experimental evidence confirming the presence of nucleosomes? Explain using the information in the gel to support your answer.

Problem 3.2.3

(tags: #nuclease digestion, #DNA packing, #gel electrophoresis, #prediction, #experimental controls, #animals)

In order to compare and contrast the chromatin of different organisms, your supervisor wants you to run a nuclease digestion experiment on salmon and chicken DNA.



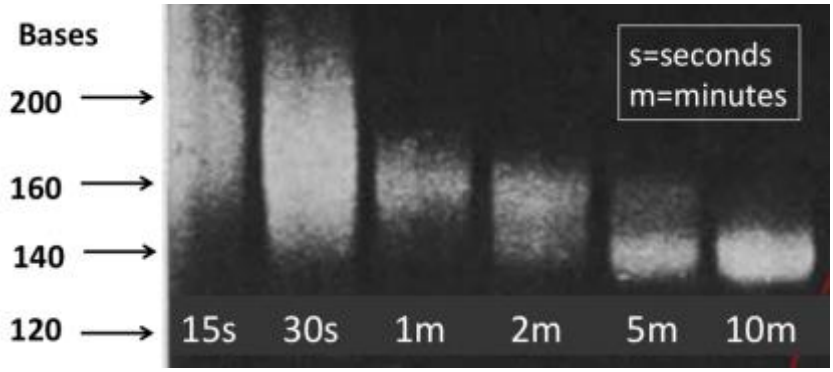
a) The final results of the comparison, along with the proper controls, require a gel with a minimum of 4 lanes. Outline what you would run in each lane. Be sure to identify which ones are controls, and what they are controlling for.

b) You run your experiment, and find that the DNA has the structure below. Draw and fully label your gel, showing what you would expect to find in each lane.

Problem 3.2.4

(tags: #DNA packing, #nuclease digestion, #gel electrophoresis, , #experimental controls)

To produce the figure below, a 240 bp fragment from a partial nuclease digestion of chromatin (DNA plus histones) is subjected to digestion by micrococcal nuclease for a longer time period. The DNA was then separated from the histones, run on a gel and viewed.



a) Describe the change in the banding pattern as time of digestion increases. What does this tell you about the size of the DNA in each lane?

b) Based on this data, what is the length of DNA present in the nucleosome core and in the linker?

c) Suggest one possible control for this experiment, and explain why this control would be necessary.

Problem 3.2.5

(tags: #DNA packing, #nuclease digestion, #gel electrophoresis, #animals)

Up until now, you have been using micrococcal nuclease in your experiments. A labmate suggests that since DNase I is also a nuclease, it could also be used in your experiments. You run the following DNA digests on rat chromatin to see if your lab mate is right:

Lane 1: no nuclease

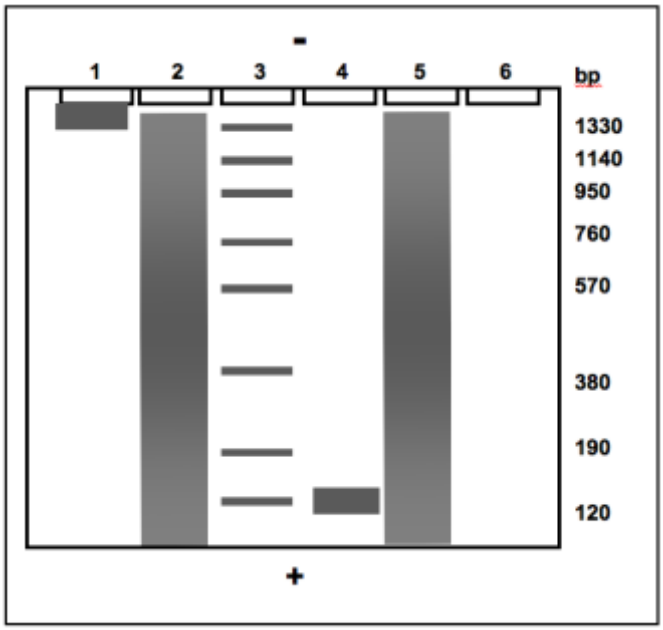
Lane 2: partial digestion of naked DNA with micrococcal nuclease

Lane 3: partial digestion of chromatin with micrococcal nuclease

Lane 4: extended digestion of chromatin with micrococcal nuclease

Lane 5: partial digestion of chromatin with DNase I

Lane 6: extended digestion of chromatin with DNase I



a) Describe the data presented in the gel.

b) Can DNase I be used to study chromatin structure? Explain your answer.

c) Based on the experimental data presented, draw a labelled "bead-on-a-string" structure for rat chromatin, including measurements. Indicate which lane(s) provided useful data for this drawing.

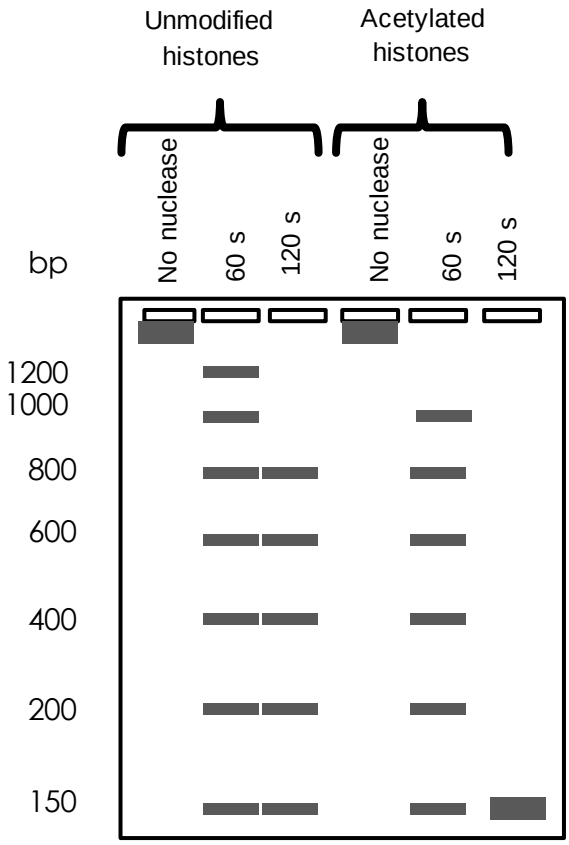
Topic 3.3 – Regulation of Gene Expression

Problem 3.3.1

(tags: #DNA packing, #gel electrophoresis, #nuclease digestion, #histones, #yeast)

Histones can be modified by the addition of acetyl groups () (termed "acetylation"), and this modification affects packaging of chromatin.

Oppikofer *et al*. (2011) conducted the following experiment to examine the effect of acetylation on chromatin structure. In this experiment, chromatin (with either unmodified or acetylated histones) was isolated and exposed to micrococcal nuclease for different periods of time (60 s compared to 120 s).



a) What is the purpose of analyzing chromatin that has not been exposed to nuclease?

b) What is the purpose of carrying out nuclease digestion experiments on DNA with unmodified histones?

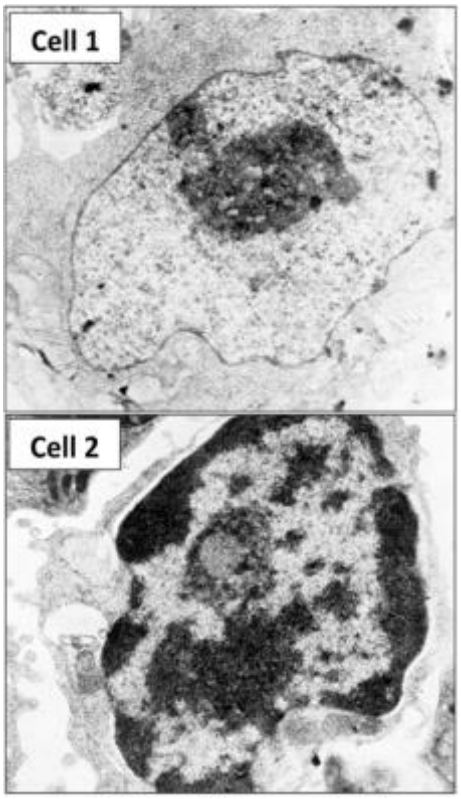
c) Describe how the DNA banding patterns change in response to the different treatments. Explain what has happened to the DNA in each lane, to give this pattern.

d) Based on this data, what can you conclude about the role of acetylation on histone function and chromatin structure? Explain.

Problem 3.3.2

(tags: #nuclear structure, #TEM, #DNA packing)

In the images on the right, cell 1 has more euchromatin than heterochromatin compared to cell 2. Which of the following statements is true? Explain your choice.



a) Cell 1 has more histones present than cell 2

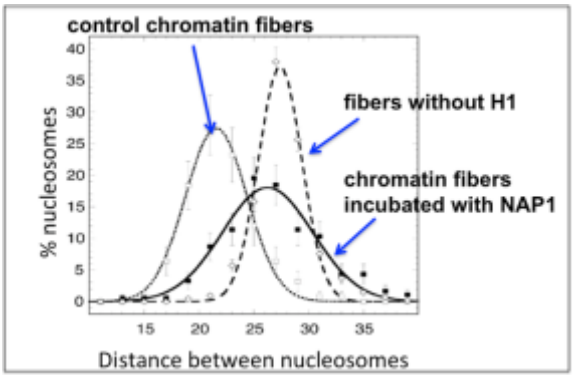
b) Cell 1 has more DNA than cell 2

c) Cell 1 is more transcriptionally active than cell 2

Problem 3.3.3

(tags: #gene expression, #DNA packing, #animal)

Chromatin is extremely dynamic; it is constantly rearranging its three-dimensional structure. Several factors, including the presence of linker histones, have been identified that promote a transition between a transcriptionally active and a denser/inactive chromatin conformation.



Researchers were interested in whether another protein (NAP1) also had a role in these rearrangements (Data from Kepert et al. (2005) J. Biol. Chem. 280: 34063-72)

a) Based on the data shown, what effects do NAP1 and H1 have on the nucleosome structure?

b) Based on this data, propose a model for how NAP1 might be involved in chromosome rearrangement.

Problem 3.3.4

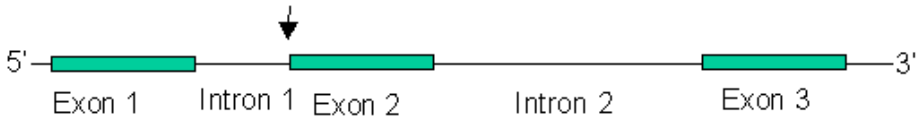
(tags: #gene expression, #mutant analysis, #RNA splicing)

The normal splice receptor sequence (at the distal (3') end of an intron) is UAG | G where the vertical slash is the end of the intron; the last G is in the exon. This sequence is absolutely required for intron excision.

a) What do you think would happen if the first splice acceptor sequence in a multiple intron protein were mutated to UAA | G?

b) What would be the consequences for the resulting protein? Explain your reasoning.

Here is a diagram that might help you visualize the situation. The arrow indicates the position of the sequence change.



Problem 3.3.5

(tags: #gene expression, #DNA packing, #essay outline)

The ability to write strong supporting arguments takes practice. In this question we provide you with the thesis statement, and you must develop 3 supporting arguments, with evidence for each one. Remember to write in full sentences, but to keep it short and concise as well.

a) Binding of proteins to DNA and RNA is the most essential process for nuclear organization and function.

b) Histone proteins have an important role to play in transcription, as they help to determine when and how the transcription factors can bind to the DNA.

c) Specific DNA sequences have the capacity to produce several different proteins, but not usually in the same cell at the same time.

Problem 3.3.6

(tags: #gene expression, #mutant analysis, #gel electrophoresis, #experimental controls, #animals)

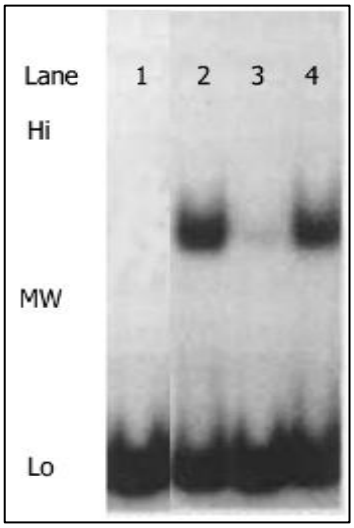
This problem is based on a classic experiment in which scientists first discovered a specific type of transcription factor (now known as the basic Helix-Loop-Helix, or bHLH transcription factors). At this point in the history of genetics, it was not well known how genes were regulated, so the discovery of proteins bearing regions that bound to specific sequences of DNA was significant. Your task in this problem is to analyze the data, and understand how the data resulted in the conclusion that we now take for granted in cell biology and genetics.

This experiment happened before the age of bioinformatics, so things like DNA sequence and protein binding had to be determined experimentally. This used a type of gel electrophoresis that did not denature the proteins, so the complex would remain intact (this is different from SDS-PAGE, in which the proteins are completely unfolded and the subunits are then separated by size). The data is from Murre et al. 1989 Cell 56, 777.

The protocol that they carried out was as follows:

- A DNA fragment thought to have a transcription factor binding site was specifically labeled using nucleotides that had radioactive ^{32}P.
- The DNA is mixed with protein and allowed time to bind, and the results are examined by non-denaturing gel electrophoresis (so the complexes stay intact in the gel).
- The protein itself is not labeled in any way. This is significant, and means that you must think carefully about what you are seeing in the gel (and what you are not!).

The image on the left shows the results of this experiment.
Each lane represents the following conditions:



1. Only labeled DNA was added to this lane. It was never exposed to the protein.
2. Labeled DNA was mixed with the predicted transcription factor prior to being run on the gel.
3. The protein was exposed to 2 types of DNA at the same time, prior to being run on the gel:
   - An excess of unlabeled DNA, that had the correct binding site (GGCAGGTG)
   - A small amount of labeled DNA, also with the correct binding site.
4. The protein was exposed to 2 types of DNA at the same time, prior to being run on the gel as follows:
   - A small amount of labeled DNA, that had the correct binding site (G**G**CA**G**GTG)
   - An excess of unlabeled DNA that had a mutation in the binding site (G**T**CA**T**GTG) for the transcription factor.

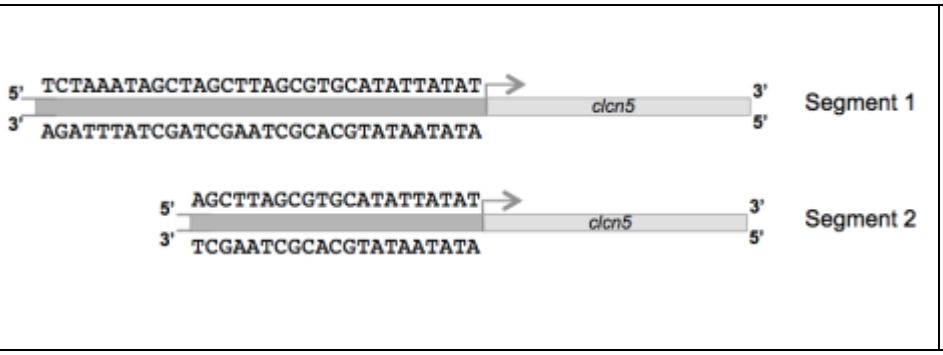
Questions:

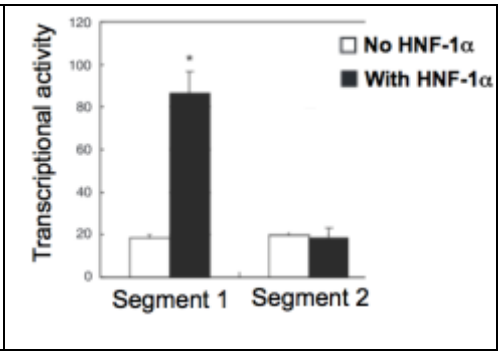
a) Can you interpret what each of the bands represents in the different lanes? What is it you can SEE in the gel? What else is there that you CANNOT see? Is it the same for all lanes?
b) Which one(s) of these lanes are controls? What do they control for? Which one(s) are the test conditions?
c) Based only on this data, what does it tell you about the specificity of transcription factor binding?

Problem 3.3.7 (Walkthrough Available)

(tags: #gene expression, #mutant analysis, #experimental controls, #prediction, #animals)

The *clcn5* gene encodes for a membrane protein that forms a chloride-proton channel in the kidney epithelium. Tanaka *et al*. (2010, Amer J Physiol Renal Physiol 299: F1339) studied how the *clcn5* gene is regulated by analyzing the transcription activity of different segments of DNA upstream of the *clcn5* gene in the presence or absence of a transcription factor HNF-1a. Below is a schematic of the gene and DNA regions they analyzed:



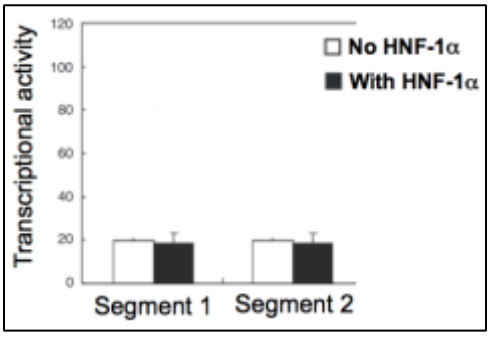


a) What is the control in this experiment? Explain why it is used.

b) Describe how the transcriptional activity of the Segment 1 and Segment 2 change when HNF-1a is present. What does this tell you about how HNF-1a controls expression of the *ccln5* gene?

c) Predict what would happen to transcription if the first 3 base pairs at the 5' end of Segment 1 were mutated from TCT to AAA. Justify your answer.

d) You are working in a lab and you decide to try and repeat the experiment from A and B. However, your data do not agree with the published results (See bar chart, right).

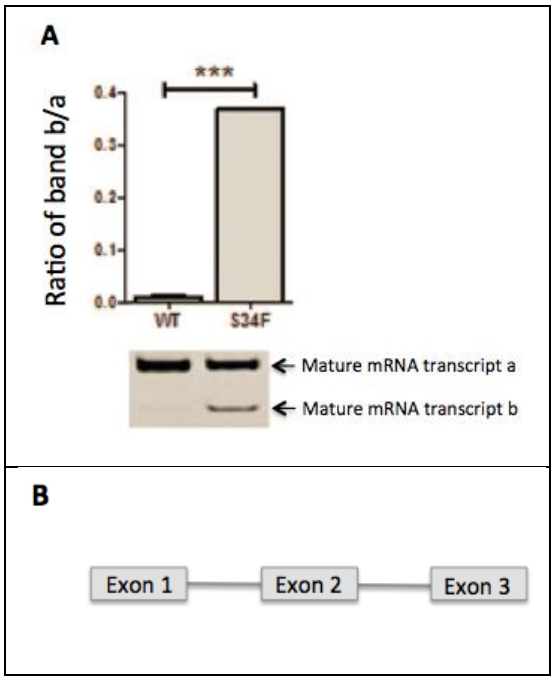


Your supervisor points out that you were accidentally using cells that lack enzymes that modify core histone proteins by adding acetyl groups. Explain why this omission could explain your result.

Problem 3.3.8

(tags: #gene expression, #mutant analysis, #gel electrophoresis, #RNA splicing, #experimental controls, #prediction, #animals)

Okeyo-Owuor et al. (2015, Leukemia 29: 909) examined how a mutation in U2AF1, a protein component of the spliceosome, affects processing of mRNA. In this experiment, mature mRNA transcripts from the DEK gene were isolated from cells expressing Wild Type (WT) and mutant (S34F) forms of U2AF1, and transcript length was examined through gel electrophoresis as shown in Box A. The diagram in Box B shows the introns (lines) and exons (boxes) that are predicted to be present in the unprocessed mRNA transcript, based on the DNA sequence.



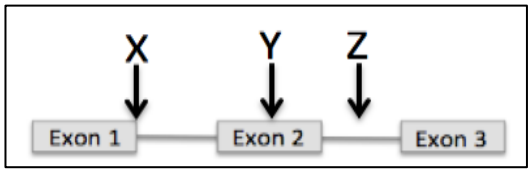
a) What is the control for this experiment? Explain why it is used here.

b) Describe the change in mRNA length in wild type and the mutant by referring to the data in Box A. Use this data to explain the role of USAF1 in DEK mRNA processing.

c) Drawn below are diagrams of primary mRNA transcripts of the DEK gene. Indicate clearly on the diagrams below which regions would be spliced out to produce transcripts a and b.

Transcript a	**Transcript b**
Exon 1 — Exon 2 — Exon 3	Exon 1 — Exon 2 — Exon 3

d) Consider a primary mRNA transcript with mutations at location X, Y, or Z, as shown below. Under normal conditions with a fully functional spliceosome, predict the impact that each mutation would have on mRNA processing and explain your reasoning.



Unit 4: Endomembranes

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 4.1 – Introduction & Protein Import

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) What are the common properties of 'start transfer' and 'stop transfer' sequences? (1)

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

f) Define the role of chaperones in protein processing. What determines whether a given protein will need chaperones for proper folding? (2)

g) How many ways can a given protein fold? What determines the folding pathway that a given protein takes, and what happens to proteins that fail to fold properly in the ER? (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)

j) How come disulfide bridges are far more common in secreted proteins than in cytosolic proteins? (3)

Topic 4.2 – 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) How do Rabs and tethers facilitate vesicle docking? (2)

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

Topic 4.3 – Golgi and Protein Processing

a) Define the following terms and determine which ones might be synonyms of each other (1):
- *Cis*-Golgi 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 4.4 – Post-Golgi Traffic

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 is sorted into the regulated or the constitutive secretory pathway? (3)

Topic 4.5 – Endocytosis

a) What is the difference between early and late endosomes? (1)
b) Define receptor-mediated endocytosis & 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)

Practice Problems

- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- Note that the problems are roughly divided by topic, but you should expect to use knowledge from other parts of the course as well.

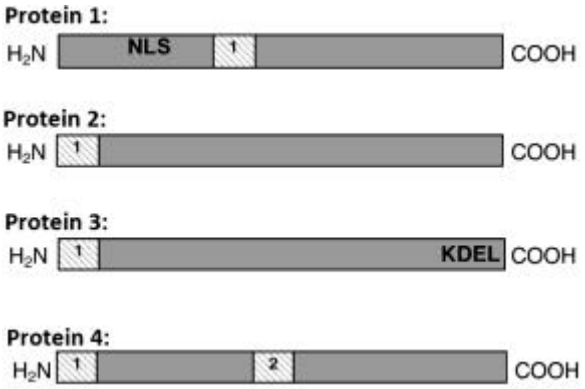
Topic 4.1 – Overview of Secretion & Protein Import

Problem 4.1.1 (Walkthrough Available)

(tags: #NLS, #hydrophobicity, #protein targeting, #protein topology, #prediction, #transfer sequence)

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.



Problem 4.1.2

(tags: #protein targeting, #KDEL, #NLS, #M6P, #prediction)

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.

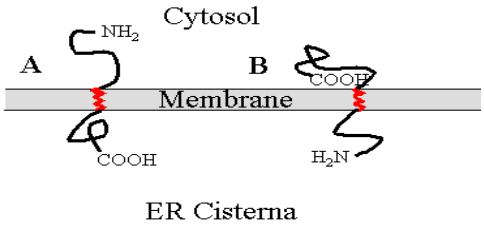
c) An N-Terminal signal sequence, and a protein sequence indicating the addition of an M6P oligosaccharide.

d) No sorting signals.

Problem 4.1.3

(tags: #protein targeting, #protein topology, #transfer sequence)

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

N – C

Protein B

N – C

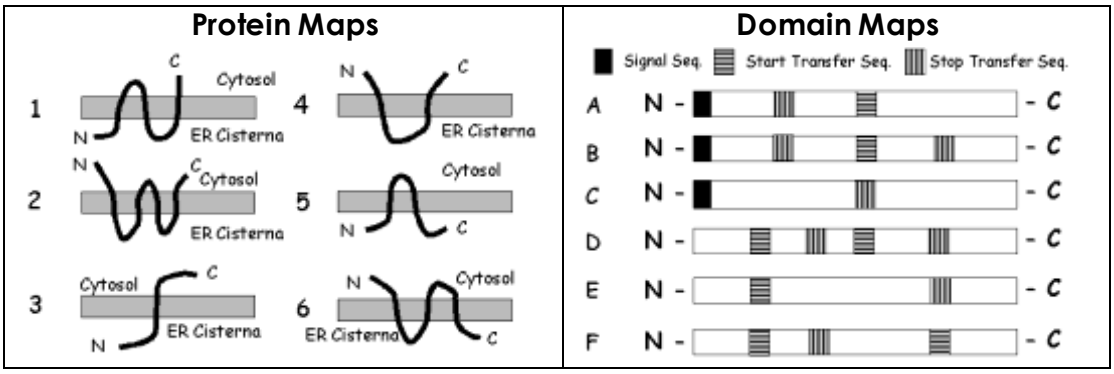
b) Make a map for the arrangement of signal sequences, start transfer sequences and stop transfer sequences for a double pass protein with the N terminal end...

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

Problem 4.1.4

(tags: #protein topology, #signal sequence, #transfer sequence)

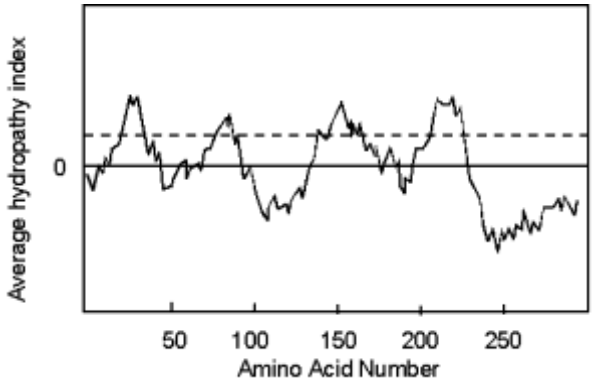
Match the following arrangements of proteins in the membrane with the appropriate polypeptide domain maps below:



Problem 4.1.5

(tags: #hydrophobicity plots, #protein topology)

The figure on the left is an average hydrophobicity 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.

Problem 4.1.6

(tags: #gel electrophoresis, #drug treatments, #protein glycosylation)

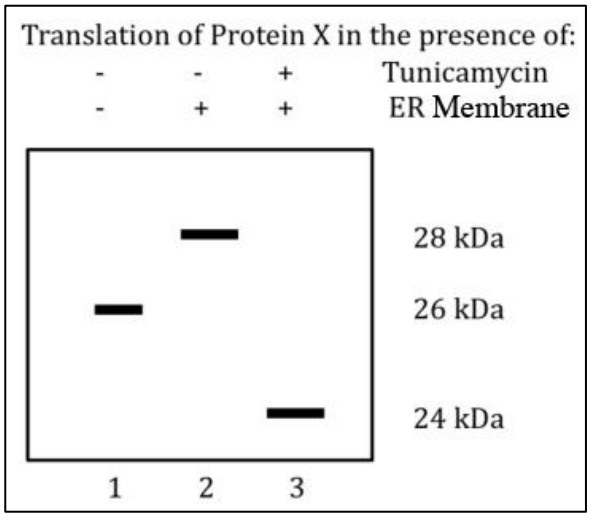
In vitro protein synthesizing systems, containing free amino acids, mRNA, and cytosolic extracts, can be used to study protein synthesis. The cytosolic extracts provide ribosomes and all cellular proteins and factors necessary for protein synthesis.

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) Describe the data for each treatment.

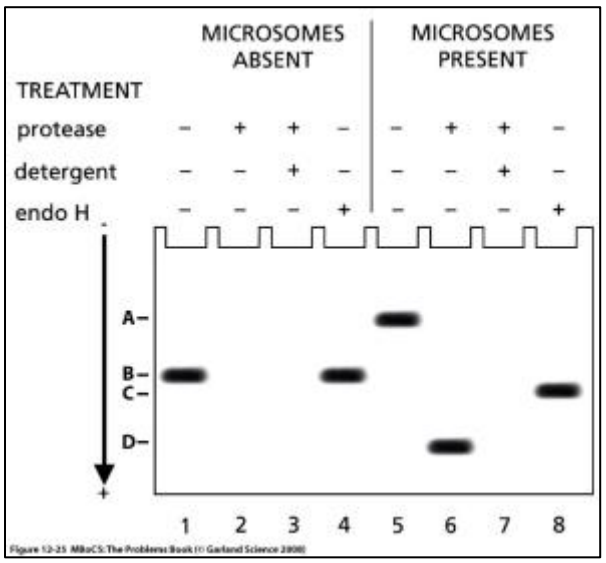
b) Explain the differences in lane 1 versus lane 2.

c) Explain the differences in lanes 1 and 2 versus lane 3.

Problem 4.1.7

(tags: #SDS-PAGE, #protein topology, #experimental controls, #protein glycosylation)

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.

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

a) Describe what you observe in each lane of the gel. Include which lanes are controls, and what they are controlling for.

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.

   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.

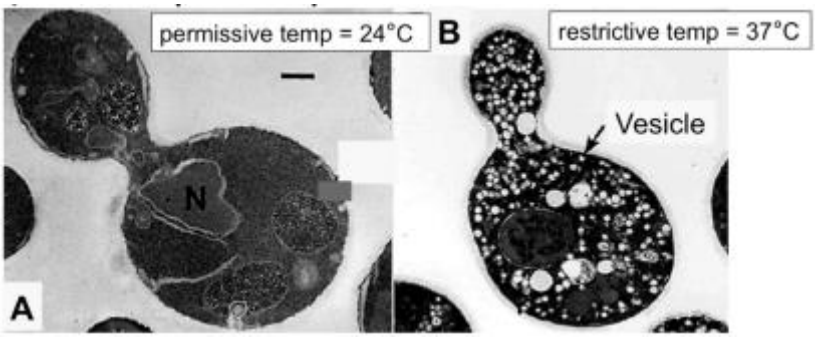
c) Is this protein an integral membrane protein? How do you know?

Topic 4.2 – Vesicle Transport

Problem 4.2.1

(tags: #mutant analysis, #temperature sensitive mutant, #TEM, #vesicle transport, #experimental controls, #yeast)

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) Describe what you observe in the test condition.
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?

Problem 4.2.2

(tags: #COPII, #mutant analysis, #fluorescence microscopy)

Explain the following experimental results:

a) A mutant cell in which the COPII 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.
(NOTE: This question will require information from Unit 6 in order to answer it).

Problem 4.2.3

(tags: #SNAREs, #vesicle transport)

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?

Problem 4.2.4

(tags: #SNAREs, #vesicle transport)

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?

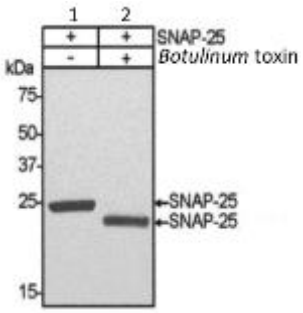
Problem 4.2.5

(tags: #SDS-PAGE, #SNAREs, #fluorescence microscopy, #plane of section, #animals)

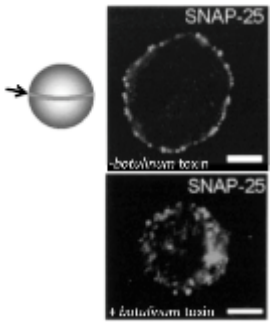
Botox® is a protease from the bacterium *Clostridium botulinum*. Low concentrations of this toxin are commonly used to eliminate wrinkles, because it prevents the secretion of acetylcholine (a neurotransmitter), thus blocking nerve impulses to muscles underneath.

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.

Panel A



Panel B



a) Panel A shows an SDS-PAGE protein gel of isolated SNAP-25 protein. First describe the change in SNAP-25 with and without *Botulinum* toxin, and then 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. Which organelle is SNAP-25 localized to when the toxin is not present? Does the localization change when the toxin is introduced? Explain.

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 4.3 – Golgi and Protein Processing

Problem 4.3.1

(tags: #protein glycosylation)

a) Are proteins in the nucleolus glycosylated? Justify your answer.

b) List all of the places in the cell that you would expect to find glycosylated proteins

Problem 4.3.2

(tags: #fluorescence microscopy, #protein targeting)

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 all the localization signals that this protein would need to reach the Golgi and stay there.

Problem 4.3.3

(tags: #drug treatments, #protein glycosylation, #lysosome targeting)

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.

Problem 4.3.4

(tags: #essay outline, #protein topology, #protein glycosylation)

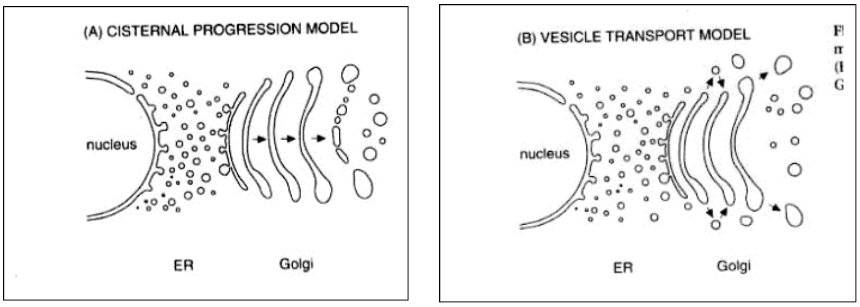
Here is a sample argument outline question for you to practice with. For this Problem, please write a thesis statement that addresses the statement below, along with 3 supporting arguments, and one piece of evidence that supports each of your arguments. Post your answer on the discussion board so you 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.*

Problem 4.3.5 (Covered in Tutorial)

(tags: #Golgi processing, #differential centrifugation, #mutant analysis, #radiolabelling, #experimental controls, #prediction, #animals)

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 (***continued on next page***)

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).

(continued on next page)

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) This experiment is testing for movement of proteins between what two Golgi compartments? Explain your answer briefly.

b) If proteins moved through the Golgi apparatus by cisternal maturation, what result would you predict for row 3 of the table above? Why?

c) If proteins moved through the Golgi apparatus by vesicle transport, what result would you predict for row 3 of the table above? Why?

d) Explain which model is supported by the results in the table? Why?

e) What false assumption do we now know that the researchers made that make their results inconclusive? Explain why this is problematic for the experimental design.

Topic 4.4 – Post-Golgi Traffic

Problem 4.4.1

(tags: #mutant analysis, #protein targeting, #prediction, #animals)

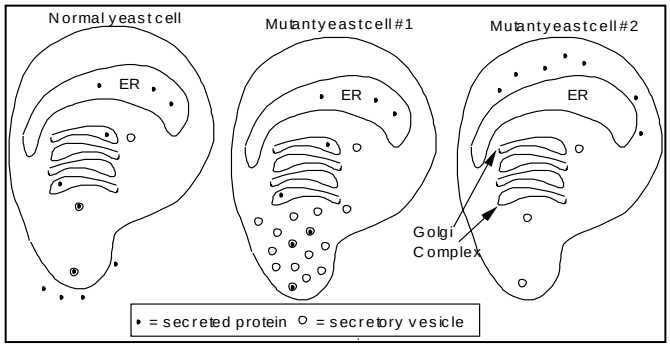
Cystic Fibrosis is a genetic disorder in which patients lack a Cl- channel (known as the CFTR protein) in epithelial cells that is important in the formation of sweat, mucus and digestive juices. As a result of the loss of this channel, patients suffer a variety of symptoms, including difficulty in digesting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

Problem 4.4.2

(tags: #mutant analysis, #protein targeting, #vesicle transport, #yeast)

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 above, determine whether it would affect a single specific protein (i.e. produced from a single gene), or all proteins that are being secreted.
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?

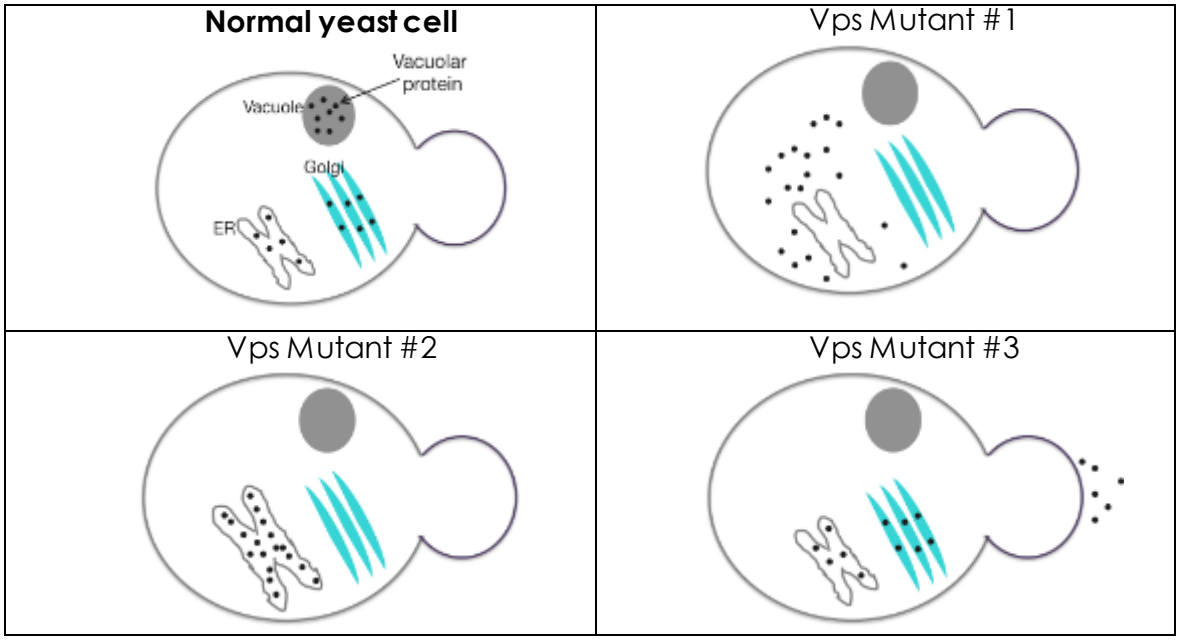
Problem 4.4.3 (Walkthrough Available)

(tags: #mutant analysis, #lysosome targeting, #yeast, #protein targeting)

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.



Problem 4.4.4

(tags: #mutant analysis, #lysosome targeting, #animals)

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?

Problem 4.4.5

(tags: #drug treatments, #secretion, #protein targeting, #lysosome targeting)

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.

Problem 4.4.6

(tags: #protein structure, #radiolabelling, #TEM, #antibodies, #animals)

Insulin is a peptide hormone that undergoes regulated secretion from 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 endomembrane 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

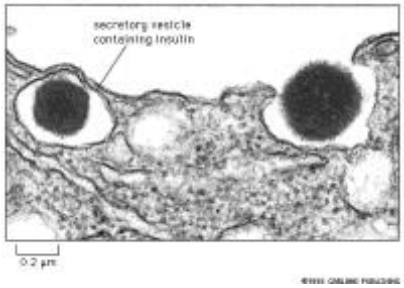
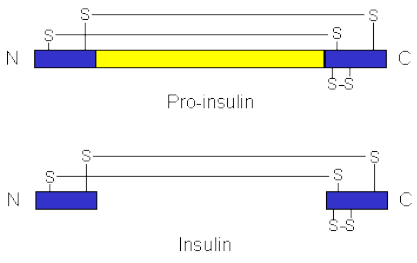


Figure 2



(Continued next page)

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.

Figure 3a: Pro-insulin antibody

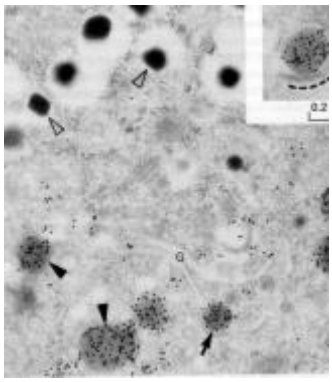
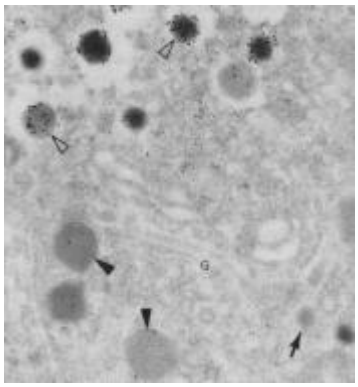


Figure 3b: Insulin antibody



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).

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.

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?

Topic 4.5 – Endocytosis

Problem 4.5.1

(tags: #endocytosis, #endosomes, #animals)

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.

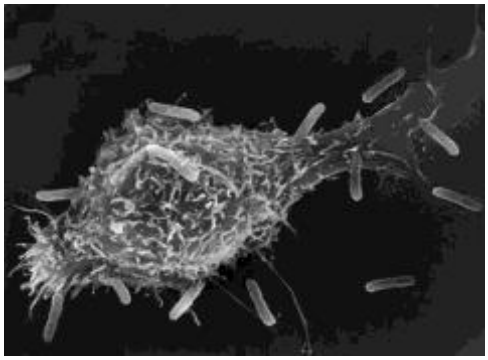
a) Where would the virus most likely end up if it doesn't "escape" the late endosome?

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.

Problem 4.5.2

(tags: #identify microscopy type, #endocytosis, #animals)



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? Why or why not?

Problem 4.5.3

(tags: #endocytosis, #endosome)

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?

Problem 4.5.4

(tags: #endocytosis, #mutant analysis, #fluorescence microscopy, #prediction, #animals)

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.

Unit 5: Mitochondria and Chloroplasts

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 5.1 – Introduction & Protein Import

a) List the major features of mitochondrial and chloroplast structure and function that are consistent with the notion that they originated from prokaryotes (1):
   - Cell surface membranes.
   - Internal membranes.
   - ribosomes.
   - Genomes.
   - Electron transport systems.
   - Formation of ATP
   - Any other characteristics that you can think of?

b) What features are shared by mitochondria, chloroplasts and bacteria that are not shared by their eukaryotic hosts? (1)

c) Compare and contrast the mechanisms of mitochondria and chloroplast import with import into the other organelles that were discussed in previous topics (2).

Topic 5.2 & 5.3 – Mitochondria and Chloroplasts

a) What is the main energy storing molecule produced by mitochondria? Chloroplasts? (1)

b) Compare the nature of the electrochemical gradient involved in generation of ATP in mitochondria with that in chloroplasts? (2)

c) Compare and contrast mitochondria & chloroplasts with regard to structure & function. (2)

d) List the main products of the mitochondrial electron transfer reactions. (1)

e) List the main products of the photosynthetic electron-transfer reactions. Why is light needed for these reactions? (1)

f) The conversion of CO_2 to carbohydrates does not directly require light energy. What necessary components does light energy **indirectly** provide for this conversion to occur? (1)

g) If chloroplasts make ATP and synthesize sugars, then why do plants need mitochondria even when growing in the light? (2)

h) Trace a molecule of oxygen from its entry into an organism, through the cell and into the mitochondria for use in oxidative phosphorylation. (3)

i) Trace energy from the sun to energy in ATP in the cytosolic compartment of a plant cell. (3)

Practice Problems

- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- 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 – Introduction & Protein Import

Problem 5.1.1 (Walkthrough Available)

(tags: #protein targeting, #KDEL, #NLS, #M6P, #signal sequence, #transfer sequence, #chloroplast targeting, #mitochondrial targeting, #CDK, #microtubules)

For each protein identified in the table below, choose from the first column the signal(s) the protein would require and write the appropriate NUMBER(S) for the signal(s) in the answer column. Note that each signal may be used more than once, not every signal mentioned will necessarily be used, and not every signal needed may be listed in first column.

Signal	**Protein**	Answer
1. Nuclear Localization Signal (NLS)	Small nuclear ribonucleoprotein (snRNP)	
2. N-terminal hydrophobic signal sequence	Chloroplast aminoacyl tRNA synthetase (gene in nuclear DNA)	
3. No signal	Mitochondrial ATP synthase (gene in nuclear DNA)	
4. Mannose 6-phosphate on oligosaccharide	M-CDK	
5. KDEL sequence	RNA polymerase III	
6. Hydrophobic transfer sequence	Na+/K+ ATPase (integral plasma membrane protein)	
7. Chloroplast N-terminal targeting sequence	Lipase (lysosomal enzyme)	
8. Mitochondrial N-terminal targeting sequence	Alpha tubulin	
	Zymogen (secreted protein)	
	Pho86p (ER resident)	
	Mannosidase (soluble Cis-Golgi resident)	

Problem 5.1.2

(tags: #nuclear structure, #mitochondrial structure, #chloroplast structure)

Chloroplasts, mitochondria, and nuclei are three intracellular organelles that are surrounded by two biological membranes. Although they possess some structural similarities, they have many differences in function and biogenesis. For statements 1 to 7 in the table below, indicate whether it applies to:

A. Chloroplasts only
B. Mitochondria only
C. Nuclei only
D. Both chloroplasts and mitochondria, but not nuclei
E. All three organelles

STATEMENT	Fill letter A-E
1) These organelles contain remnant genomes carried on circular chromosomes, which reflect their evolutionary origins.	
2) mRNA is transported through multi-protein complexes in the membranes surrounding this organelle to the cytoplasm for translation.	
3) This organelle contains euchromatin and heterochromatin.	
4) The outer envelope membrane is continuous with the endoplasmic reticulum.	
5) The outer envelope membrane, but not the inner envelope membrane, is porous and allows molecules to diffuse across freely.	
6) The inner envelope membrane of this organelle is highly folded increasing its surface area.	
7) This organelle contains an additional internal membrane system, in addition to the two membranes surrounding the organelle.	

Problem 5.1.3

(tags: #chloroplast targeting)

State whether you think the following statement is true or false, and explain why:

If a chloroplast protein could be removed from the chloroplast after import, it would be returned to chloroplast by import proteins in the cytosol.

Problem 5.1.4

(tags: #secretion, #lysosome targeting, #chloroplast targeting)

Compare and contrast the pathway of biosynthesis of an N-glycosylated lysosomal enzyme with an enzyme used in the Calvin cycle from the beginning of its synthesis on a free ribosome in the cytoplasm (excluding the details of translation) to its final intracellular destination. Be as specific as you can.

Problem 5.1.5

(tags: #endosymbiosis, #mitochondria, #animals, #plants)

This problem explores the mitochondrial genome and what it might tell us about its origins.

The figure on the right (Fig 14-55, MBoC, 4th ed.) compares the sizes of mitochondrial genomes with that of its closest bacterial relative (Rickettsia sp.).

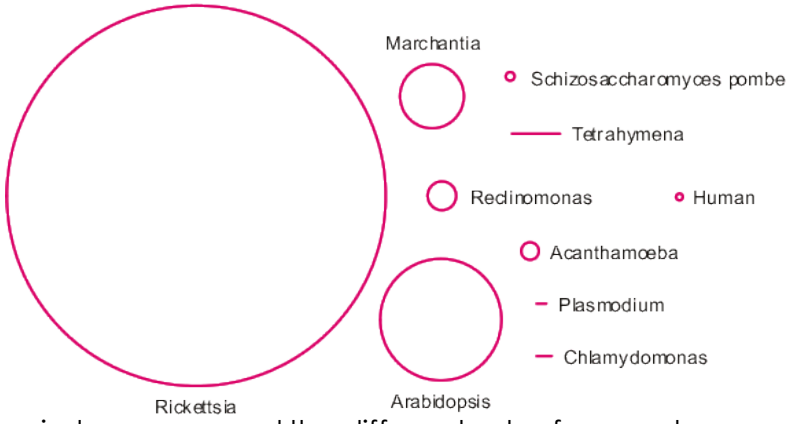
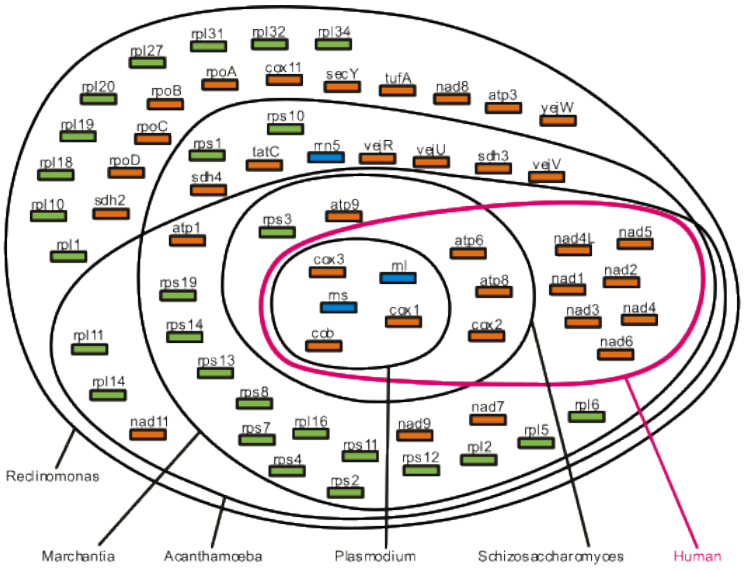


Figure 14-57 (below, MBoC, 4th ed.) shows the full suite of known mitochondrial genes and how different subsets of these genes are present in different organisms. Much like a Venn diagram the circles are around the different sets of genes show wich genes are present in which species. Notice that there is a subset of genes (the smallest circle) that is present in all mitochondrial genomes.

Finally, this figure (Fig 14-58, MBoC, 4th ed.) is a map of the human mitochondrial genome. This genome encodes about 11 proteins.



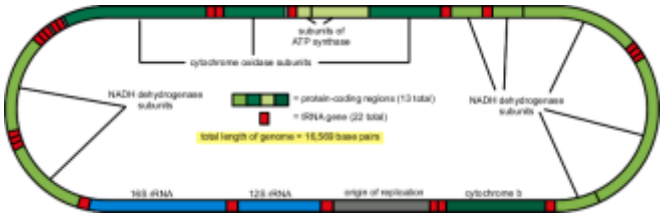
Questions:

a) Why do you think that the mitochondrial genomes are so much smaller? Do you think that mitochondria contain only a small fraction of the number of different proteins that a bacterium does? Where do you think that the genes went?

b) Does the data in figure 14-57 suggest that there are one or several independent origins of mitochondria? Why or why not?

c) Where in the phylogenetic tree of eukaryotes do you think that mitochondria originated? Why? Is the information in figure 14-57 any help? What assumptions do you have to make?

d) Where do the rest of the proteins in a mitochondrion come from?



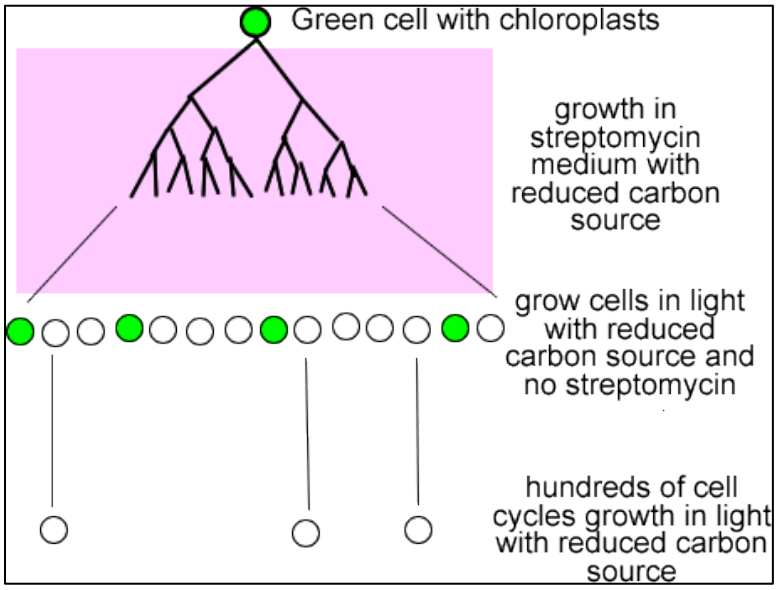
Problem 5.1.6

(tags: #chloroplasts, #endosymbiosis, #drug treatments, #plants)

Note about choosing a subject for experimental studies on chloroplast continuity and development: *Euglena sp.* is an excellent organism for studies of chloroplast continuity because it is a facultative heterotroph, meaning that in the absence of light it can grow in medium containing reduced carbon compounds (e.g. sugars). This means that if, for some reason, the cell loses its chloroplasts it can continue to grow as long as there are carbohydrates in the growth medium. In addition, if cells with chloroplasts have been grown in the dark, the cells may not look like they have chloroplasts (i.e. they won't be green) but the chloroplasts and the entire photosynthetic apparatus redevelop when the cells are returned to the light. In the dark plastid continuity is provided by a related organelle known as a proplastids, which become chloroplasts in the presence of light.

On the other hand, blocking chloroplast reproduction, but allowing continued growth and division of cells results in loss of chloroplasts in some lines of descent. Streptomycin is an antibiotic that binds specifically to ribosomes in the chloroplast, which has the effect of inhibiting translation.

If Euglena gracilis are exposed to streptomycin for several generations of growth, and then the streptomycin is removed to allow translation to restart, only a small fraction of the cells are capable of recovering full chloroplast function.



Questions:

a) Why do some cells recover photosynthesis after streptomycin treatment and others don't?

b) Why do those that don't form green colonies immediately after streptomycin treatment never develop photosynthetic activity at later times?

Topic 5.2 & 5.3 – Mitochondria and Chloroplasts

Problem 5.2.1 (Covered in Tutorial)

(tags: #essay outline)

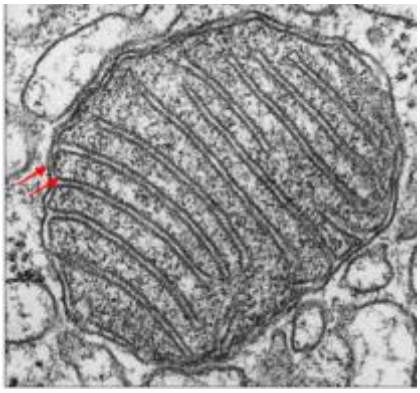
Here is a sample argument outline question for you to practice with. For this Problem, please write a thesis statement that addresses the statement below, along with 3 supporting arguments, and one piece of evidence that supports each of your arguments. Post your answer on the discussion board so you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

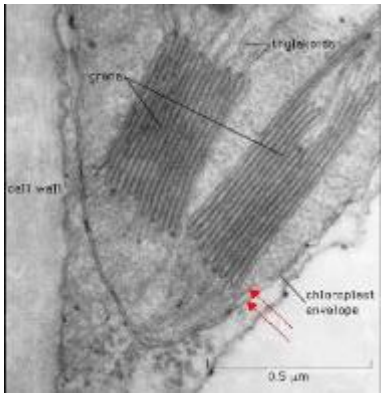
> *Each organelle in the cell is a unique microenvironment where certain cellular functions are performed. Write an essay outline to critically assess the following statement: "The intracellular areas of low pH are essential to the function of some organelles." Your arguments (and associated supporting evidence) should provide examples from 3 different organelles.*

Problem 5.2.2

(tags: #TEM, #mitochondrial structure, #chloroplast structure, #structure-function)

You can see in these micrographs that both mitochondria and chloroplasts have many folded internal membranes. Why is this feature important for the function of these organelles?





Problem 5.2.3

(tags: #mitochondria, #chloroplasts, #structure-function)

For each of the observations below, write a statement that could explain these results, based on what the data show and on your knowledge of cellular structure/ function (3 marks each)

a) Electron micrographs show that mitochondria in heart muscle have a much higher density of cristae than mitochondria in skin cells.

b) In chloroplasts, protons are pumped out of the stroma across the thylakoid membrane, whereas in mitochondria, they are pumped out of the matrix across the inner membrane. This arrangement allows chloroplasts to generate a steeper proton gradient across the thylakoid membrane.

Problem 5.2.4

(tags: #mitochondria, #chloroplasts, #structure-function)

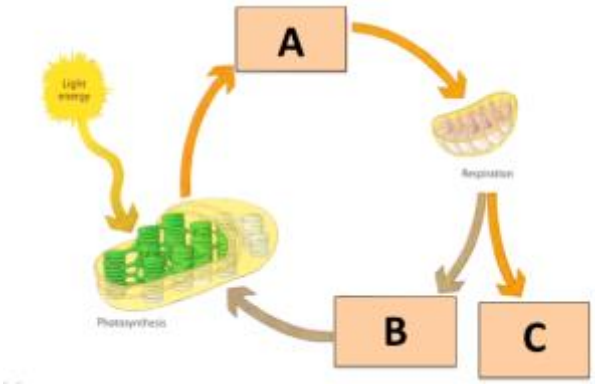
Make a diagram of a chloroplast and a mitochondrion, and label the diagram(s) with the following terms and structures.

- lower pH compartment
- compartment where ATP synthesis occurs
- Site of NADH oxidation
- Site of NADPH use in carbohydrate synthesis
- Mitochondrial electron transport chain
- Photosystem I and II
- Calvin Cycle Enzymes
- Chlorophyll
- Citric Acid Cycle Enzymes
- Site of organelle DNA
- Pyruvate transporter
- ATP export

Problem 5.2.5

(tags: #mitochondria, #chloroplasts)

Mitochondria and chloroplasts are very closely linked in the ecosystem. The products of one become the reactants of the other. Identify the molecules that should be placed in each of the boxes to complete this image.



Problem 5.2.6

(tags: #mitochondria, #chloroplasts, #electron transport chain, #proton pumps)

Protons move in both directions across in both the mitochondria and chloroplast. They move in one direction as the result of the electron transport chain.

a) Name where the electron transport chain pumps protons from and to in each of these organelles.
b) How do the protons get back to their original compartment?

Problem 5.2.7

(tags: #proton pumps, #mitochondria)

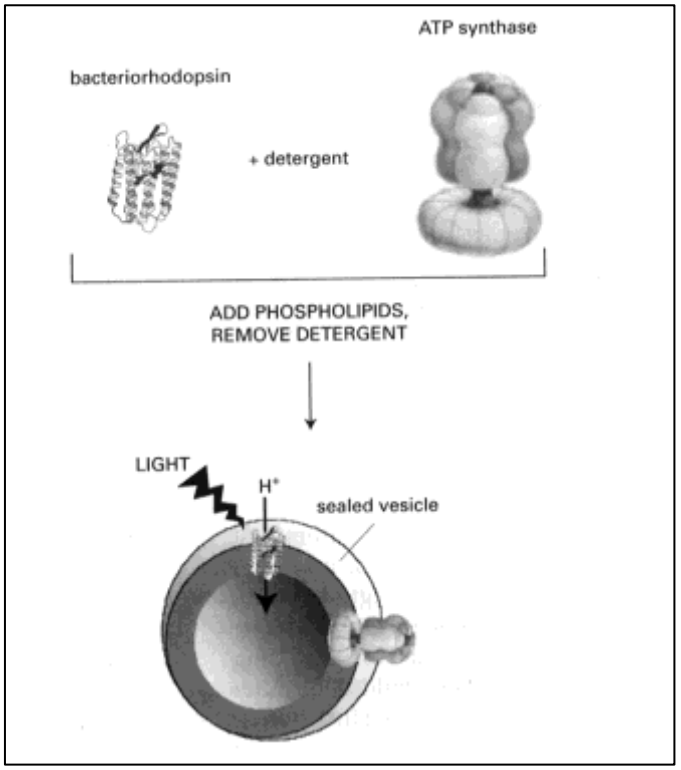
Typically, hydrogen ions flow down their concentration gradient to drive the reaction of ADP → ATP.

a) What protein do the H^+ ions flow through?
b) What would occur if the intermembrane space became substantially more basic than the matrix of the mitochondria?

Problem 5.2.8

(tags: #ATP synthesis, #prediction)

In order to study ATP production, you produce artificial phospholipid 'vesicles' (known as liposomes) in which you have incorporated two protein complexes (as shown in the image on the right):



- Bacteriorhodopsin - a light driven proton pump from purple photosynthetic bacteria.
- ATP synthase from beef heart mitochondria.

You run an experiment to explore the synthesis of ATP and make liposomes containing the following combinations of molecules:

1. Bacteriorhodopsin alone
2. Beef ATP synthase alone
3. Both proteins together

You then add ADP and Pi to the medium external to the liopsomes and shine light on them.

Questions:

a) Predict what will happen in each of the liposomes that you created and explain why.

b) If ATP is produced would it be inside the vesicles or in the external medium? Explain.

c) Which of your liposomes mimic the conditions in the mitochondria and which ones mimic the chloroplast. Explain.

d) What would happen if you added an uncoupler to these liposomes? This compound permeabilizes membranes for protons.

e) What could you add to liposome #2 to change your results in relation to ATP synthesis? Why would that work?

f) What would happen if you accidentally spilled dish soap in your experiment?

Unit 6: Cytoskeleton

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 6.1 – Introduction & Intermediate Filaments

Fill out the following table to compare and contrast the different components of the cytoskeleton. Be as specific and detailed as you can (1):

Topic…	7.1 (intermediate Filaments)	7.2 (Microtubules)	7.3 (Actin filaments)
Filament?			
Monomer?			
Structural Polarity (Y/N)			
Dynamic Instability (Y/N)			
Energy source?			
Motor Proteins?			
Function(s)?			

a) Describe the organization of intermediate filaments going from the level of single polypeptides to the microscopically visible filaments that are found in cells. (1)
b) Explain how the structure of intermediate filaments contributes to its strength. (2)
c) How do intermediate filaments contribute to the integrity of epithelial tissues? (2)
d) Compare and contrast nuclear lamins with other known intermediate filaments, such as keratin or vimentin? Make a list that includes, structure, function and distribution of these different types.
e) Thought Problem: There are no known motor proteins that use intermediate filaments as substrate. Why might that be? (2)
f) Compare the organization of intermediate filaments and chromatin. What can you say concerning how one goes from molecules to microscopically visible structure? (3)

Topic 6.2 – Microtubules

a) Explain how tubulin dimers interact non-covalently to make microtubules with a 'plus' and a 'minus' end? (1)
b) What is meant by polarity in microtubule structure? Explain what is meant by the 'plus' and 'minus' end of the microtubule (hint: It has nothing to do with charge) (2)
c) What are nucleation sites, why are they needed, and what is their role in microtubule formation? (2)
d) What are microtubule organizing centres (MTOCs)? What do they do? (1)
e) What does dynamic instability mean? How is GTP involved? (2)
f) Explain how the different microtubule motor proteins work, and how their movement relates to the polarity of the microtubule. (2)
g) Draw and label a flagellum (or a cilium), and explain how each of the proteins in it contributes to flagellar function (2).
h) Describe how microtubules are arranged in animal cells, and how their arrangement contributes to the structure and function of the endomembrane system. How might motor proteins contribute? (3)

Topic 6.3 – Actin Filaments

a) Compare and contrast the polymerization of actin with that of microtubules. (2)
b) Compare and contrast the motor proteins associated with microtubules and actin microfilaments with regard to general properties, structure and function. (2)
c) Explain how animal cell motility is dependent on the dynamic instability of actin filaments. (2)
d) Describe how associated proteins modify the function of both actin filaments and microtubules. (2)
e) Drugs are often used to disrupt normal cytoskeletal function in experiments. Explain how this might be used to learn about actin and/ or microtubule function. (3)

Practice Problems

- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- 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 6.1 – Introduction & Intermediate Filaments

Problem 6.1.1

(tags: #intermediate filaments, #actin, #microtubules)

a) List as many ways as you can come up with that Intermediate filaments are different from microtubules.

b) List as many ways as you can come up with that Intermediate filaments are different from actin filaments.

c) Do the same as a) and b), only this time list as many ways as you can think of that they are the same.

Problem 6.1.2

(tags: #intermediate filaments, #actin, #microtubules)

a) True or False: Skin epithelial cells contain a higher density of intermediate filaments than sperm cells? Explain your reasoning.

b) What cytoskeletal component would you expect to be most prominent in sperm cells? Explain why.

c) Think of a cell type whose primary function depends on the third cytoskeletal component (i.e. not the one discussed in epithelial and sperm cells) and discuss its relevance.

Problem 6.1.3

(tags: #drug treatments, #actin, #microtubules, #intermediate #filaments, #locomotion, #animals)

What do these observations tell you about the potential involvement of each of these cytoskeletal components on fibroblast locomotion? Provide an explanation for all 3 filament types.

Treatment	Result	Explanation
Cytochalasin (caps AF)	Fibroblasts freeze in place	
Colchicine (depolymerizes microtubules)	Fibroblasts stop moving directionally and start to extend lamellipodia in all directions	
Antibodies against intermediate filaments (inhibits IFs)	No change in fibroblast locomotion	

Problem 6.1.4

(tags: #intermediate filaments, #mutant analysis, #animals)

One of the components of polyacrylamide gels (used in SDS-PAGE) is acrylamide. Acrylamide is a well-known and very potent neurotoxin, though its exact mechanism is not well understood.

One hypothesis is that acrylamide causes the depolymerization of neurofilaments (a type of IF), which destroys neurofilament bundles in nerve cells. You want to test this theory, and decide to compare the effects of acrylamide on wild type mice and knockout mice that are lacking neurofilaments (interestingly, these mice have no other obvious defects).

Your results show that acrylamide is an equally potent neurotoxin in both normal and knockout mice.

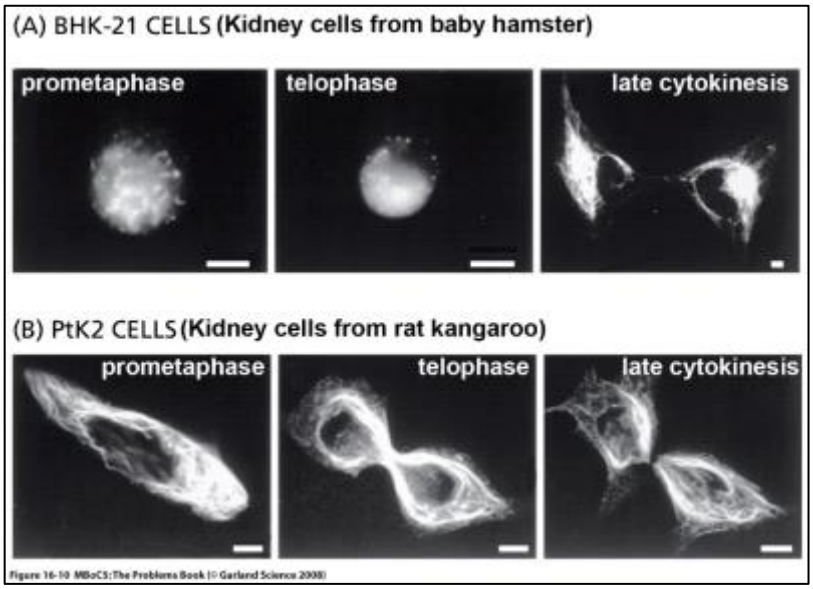
a) Discuss the general role of neurofilaments in nerve cells.

b) Do your results support the original hypothesis? Why or why not?

Problem 6.1.5

(tags: #intermediate filaments, #cytokinesis, #fluorescence microscopy, #animals)

During cell division, all components of the cell must be equally divided between the two daughter cells, including the network of intermediate filaments in the cell. However, the exact mechanism by which a cell will achieve this will differ from species to species. The figure shows the network of intermediate filaments known as vimentin, in cells of two different species of mammals as they undergo cell division, visualized by GFP-tagging and fluorescence microscopy. In all cases the scale bar is 5 μm.



a) Examine the distribution of the fluorescence at prometaphase in each species? How do they compare to each other? How about at telophase?

b) Now examine the fluorescence pattern at late cytokinesis in each species. How do they compare to each other? How do they compare to the distribution of fluorescence in the previous stages?

c) Based on these images, explain how each of the different cell types manage to successfully divide its vimentin network into the two daughter cells.

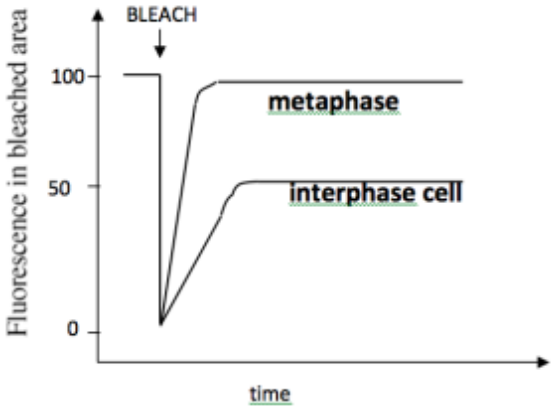
d) Despite how it may appear, cell shape is not an important issue when interpreting this particular data. Why might that be?

Topic 6.2 – Microtubules

Problem 6.2.1 (Walkthrough Available)

(tags: #microtubules, #FRAP, #cell cycle)

One approach to characterize microtubule assembly and disassembly involves injecting fluorescently-labeled tubulin into cells. The fluorescent tubulin is then incorporated into microtubules, thereby allowing visualization of the microtubules inside living cells. When these cells are used in FRAP (fluorescence-recovery-after photobleaching) experiments, the following results are found for cells that are in interphase and cells that are in metaphase.



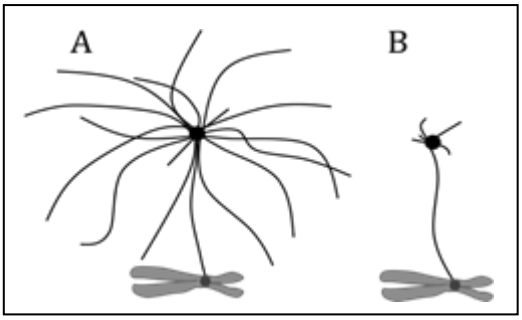
a) When doing FRAP in cells with labeled microtubules, why does the fluorescence intensity in the bleached area recover (i.e. it does not stay at zero)?

b) What can you conclude about the relative stability of the microtubules in metaphase cells as compared to the microtubules in cells in interphase? Interpret these results in terms of the function of microtubules in metaphase.

Problem 6.2.2 (Walkthrough Available)

(tags: #microtubules, #mitosis, #critical concentration)

A classic research study clearly demonstrated the properties of microtubules of the mitotic spindle. Centrosomes were used to initiate microtubule growth *in vitro*, and then chromosomes were added. The chromosomes bound to the free ends of the microtubules, as shown in Fig. A. The mixtures were then diluted to a level below the critical concentration of tubulin and examined again (Fig. B).



a) Describe what happens to the microtubules in A and B.

b) How can we account for the difference between what happens to the kinetochore microtubule and what happens to the other microtubules?

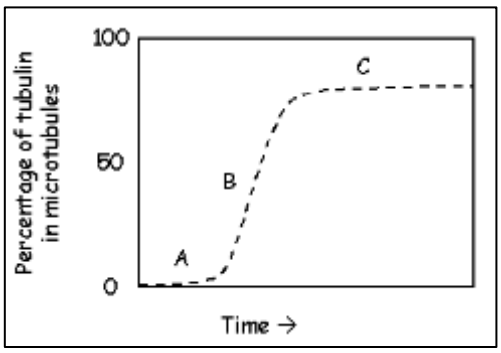
Problem 6.2.3

(tags: #microtubules)

The graph shows a typical time course of polymerization of purified tubulin to form a microtubule.

a) Explain the different parts of the curve (A, B, & C)

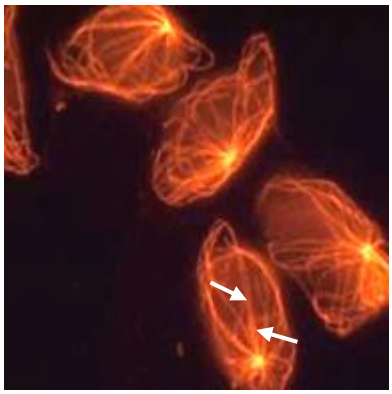
b) Identify which part of the curve is affected by the addition of nucleating sites and how the curve would change.



Problem 6.2.4

(tags: #microtubules, # identify microscopy type, #motor proteins, #drug treatments, #cell cycle, #cytoskeleton polarity, #animals, #plants)

The image on the right is of several cells in which microtubules have been labelled.



a) What kind of microscopy is this? How do you know?

b) Which motor protein would transport a vesicle from one arrow to the other? Would it be the same motor protein if the transport vesicle were moving in the opposite direction? Explain.

c) What would happen to this vesicle if these cells were treated with taxol? What about colchicine? What about latrunculin?

d) What is the bright spot that can be seen in each cell? Based solely on this bright spot, can you tell whether this is a plant or animal cell at interphase? Explain.

Problem 6.2.5

(tags: #microtubules, #drug treatments, #motor proteins, #brightfield microscopy, #cytoskeleton polarity, #animals)

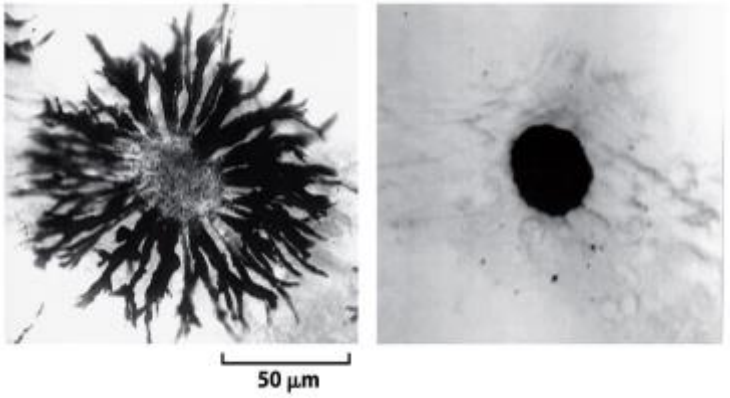
Small granules containing pigment in the epidermal cells of African cichlid fish (shown right) can aggregate or disperse in response to certain cues (below). When colchicine is added to cells in which the pigment granules have been induced to aggregate, the granules cannot disperse again.

a) Describe the results in the two micrographs.

b) What protein is most likely to be involved in aggregating the granules? Why?

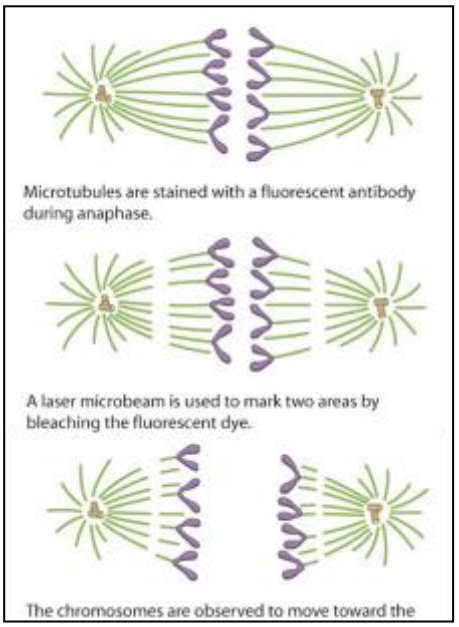
c) How could the granules be made to dispersed again? Explain your answer.

Dispersed granules (left), aggregated granules (right)



Problem 6.2.6

(tags: #microtubules, #FRAP, #mitosis, #drug treatments, #dynamic instability)



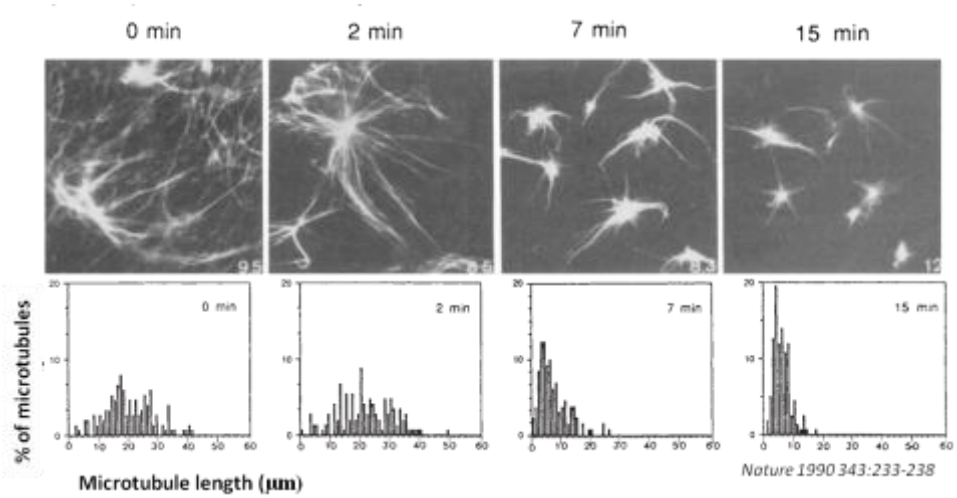
It is possible to mark the MTs of a spindle by photobleaching with a laser microbeam (see figure right). When this is done, chromosomes move toward the bleached area during anaphase.

a) Describe the data for chromosomes and for the MTs in the diagram.
b) You have learned that disassembly of MTs causes chromosomes to move to opposite poles during anaphase. Based on the data above, disassembly of MTs at which end is causing chromosomes to move to the poles? How would the data be different if MT disassembly were to occur at the other end?
c) What would be the consequence of adding taxol to this experiment?
d) What would be the consequence of adding colchicine to this experiment?

Problem 6.2.7

(tags: #microtubules, #fluorescence microscopy, #mitosis, #centrosomes, #dynamic instability, #animals)

Verde et al. (1990) studied the role of Cdc2 in mitosis. In this experiment, microtubules were formed *in vitro* by adding centrosomes to interphase cell extracts (containing tubulin). After microtubules were formed, Cdc2 was added (time = 0 minutes), and microtubules were fixed and stained with fluorescent antibodies at the indicated time points. Microtubule length was also measured at each time point.



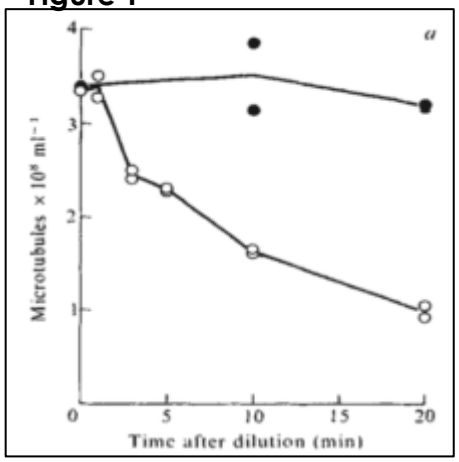
a) Why did the researchers need to add centrosomes to the cell extracts in order to study microtubule assembly?
b) Describe the results of this experiment.
c) What can you say about the role of Cdc2 in MT dynamics based on the data shown?
d) Why might the activity of Cdc2 be important for mitosis?

Problem 6.2.8 (Covered in Tutorial)

(tags: #microtubules, #TEM, #critical concentration, #dynamic instability)

Prior to the development of live cell imaging techniques, researchers had to find creative ways to study dynamic processes. Some of the earliest work on dynamic instability came from *in vitro* experiments that used electron microscopy to analyze microtubules that had been allowed to polymerize in a test tube at different concentrations, prior to visualization of static images and statistical analysis. The data presented here is some of the earliest work that was done on this subject (Original Paper: Mitchison & Kirschner 1984. *Nature* 312: 237).

Figure 1

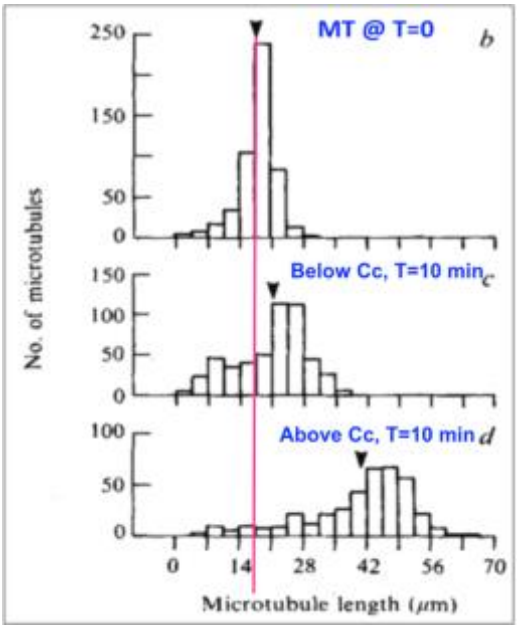


In this experiment the same concentration of short microtubules were added to 2 separate solutions of free tubulin: in the first one, the free tubulin concentration was above the critical concentration, and in the other, the tubulin concentration was below the critical concentration (note that the critical concentration had been previously determined by this same research group). The concentration of microtubules was measured over time.

Figure 1 shows the number of microtubules per ml as a function of time. Closed circles (•) are data points from the solution that was above critical concentration, while open circles (**O**) are data points from the solution that had been diluted below the critical concentration.

a) For each case, describe the change in microtubule concentration.
b) This graph only tells you about **one** property of the MTs in solution (the concentration of MTs over time). List some other properties of the MTs missing from this data.
c) Can this data tell you why is there no change in microtubule concentration above Cc? Explain your reasoning.

Figure 2



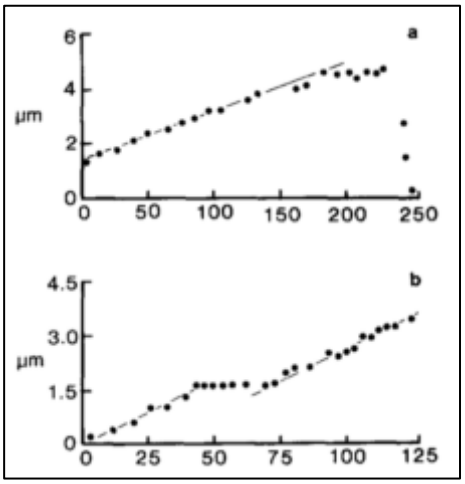
In Figure 2 (right) the researchers examined three time points shown in Figure 1 in more detail. They chose 500 microtubules at random to measure using TEM, from 3 of the samples. These histograms show a distribution of microtubule lengths at each time point. The arrowhead at the top of the histogram identifies the mean length in each sample. A line has been drawn (pink) from the top sample across the other 2 histograms to help you compare.

d) For each case, describe the change in MT length.
e) Are all of the microtubules <u>below the Cc</u> behaving the same way? Justify your answer by referring to the data from both Fig 1 and Fig 2.
f) What conclusion(s) can you draw about the relationship between microtubule concentration, microtubule length, and free tubulin concentration? Support your conclusion by explaining what is happening to the MTs in this experiment above and below the Cc. In your explanation, identify what data helped you come to your conclusion(s).

Problem 6.2.9

(tags: #microtubules, #brightfield microscopy, #dynamic instability, #animals)

Live-cell imaging and video microscopy started to become more common practice in the 1980s. This breakthrough changed the kinds of data that researchers could collect and analyze. One of the processes that really benefitted from this type of analysis was dynamic instability. Here is some data that was collected in the 1980s that contributed a great deal to how we view dynamic instability today.



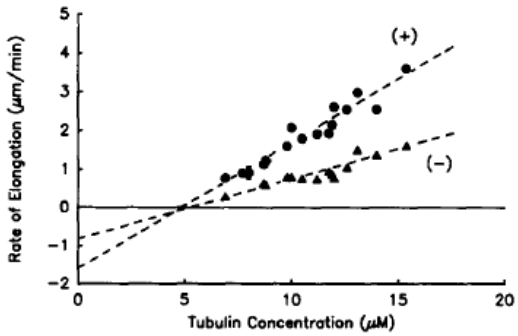
In this experiment, axonemes were isolated from flagellated sea urchin cells and visualized by light microscopy (more specifically DIC imaging). The axonemes were mixed with varying concentrations of free tubulin and allowed to polymerize/depolymerize naturally, while being recorded. These videos were analyzed statistically and the data was graphed. (Original article: Walker *et al*. (1988) *J Cel Biol* 107: 1437-1448).

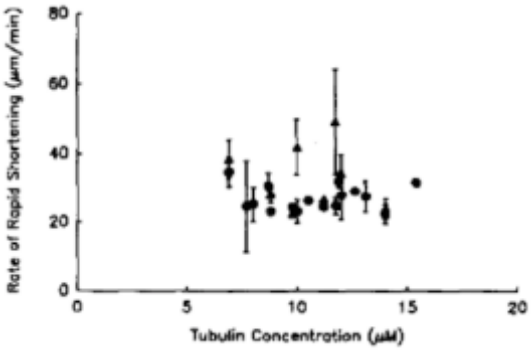
a) Describe the change in microtubule length (μm) over time (sec) in panels a and b, in the graph on the left. Note that in each case it was the plus end that was followed.
b) Do either of these microtubules depolymerize from the plus end? How do you know?
c) What happens in the middle of the data collection in panel b? What might that say about local concentrations of free tubulin versus polymer tubulin concentrations at that point?

Problem 6.2.10

(tags: #microtubules, #monomer concentration, #dynamic instability, #cytoskeleton polarity)

In the same paper that was described in the previous Problem, the rate of elongation and shortening were plotted as a function of increasing tubulin concentration. The graphs are below (elongation rate vs [tubulin] on the left, shrinkage rate versus [tubulin] on the right). In each case, the triangles refer to data collected at the minus end, and circles refer to data collected at the plus end.





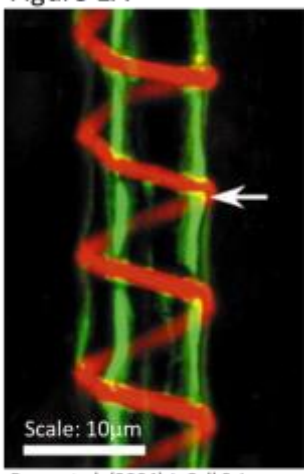
a) Which end of the microtubule has a higher affinity for free tubulin units? How do you know?
b) There's something very important to notice about the data collection versus the line of best fit in the elongation graph. What is it? How is it related to what's being shown in the second graph?
c) What to do each of these graphs tell you about the role of microtubule concentration in the growth and shrinkage of microtubules?

Problem 6.2.11

(tags: #microtubules, #plants, #identify microscopy type, #dynamic instability)

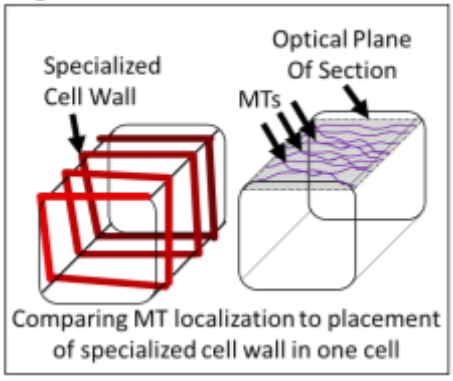
While all plant cells make a cell wall, some plant cells go on to make additional specialized cell walls with special properties. Xylem cells, which are important for water transport in vascular plants, form a specialized cell wall arranged in a spiral structure in the extracellular space (as labelled in red in Figure 1). Watanabe et al. (2015, Science 350: 198) looked at how the plant microtubule network changes when plants begin making these spiral cell walls. In plant cells, microtubules are found just under the plasma membrane (as labelled in Figure 1B). The images in Figure 2 show the fluorescently-tagged microtubules just under the plasma membrane of a single cell undergoing this transformation. The white bracket in Figure 2B indicates one of the regions where the specialized cell wall spiral is forming.

Figure 1A



Ryser et al. (2004) J. Cell Sci

Figure 1B



a) Describe the data in Figure 2 by comparing the microtubule localization in the two panels.

b) Explain why both polymerization and depolymerization of microtubules would be necessary for the change seen in microtubule localization in Figure 2.

c) Vesicles carry necessary components for the specialized cell wall to the plasma membrane. Given this information, and the data in Figure 2, why might the changes seen in the microtubule network occur?

Figure 2A

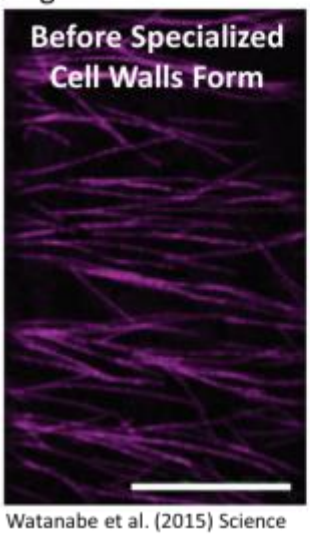
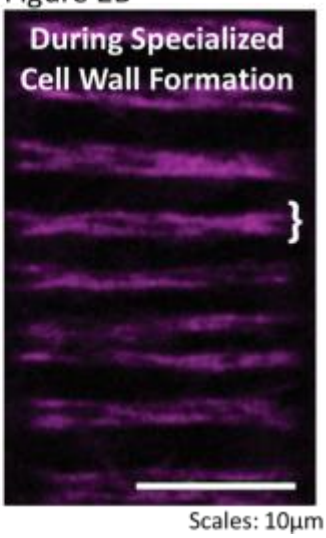


Figure 2B



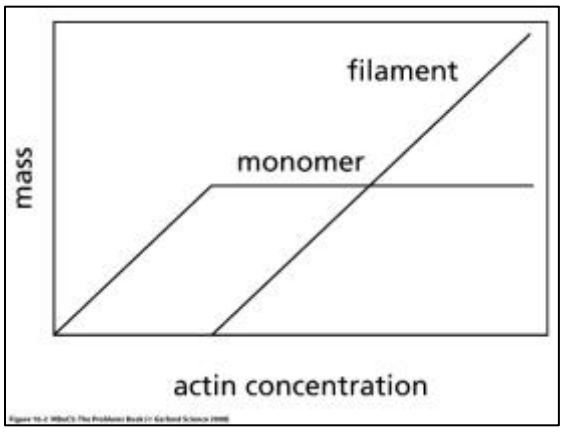
Watanabe et al. (2015) Science

Scales: 10μm

Topic 6.3 – Actin Filaments

Problem 6.3.1

(tags: #actin, #critical concentration, #monomer concentration)



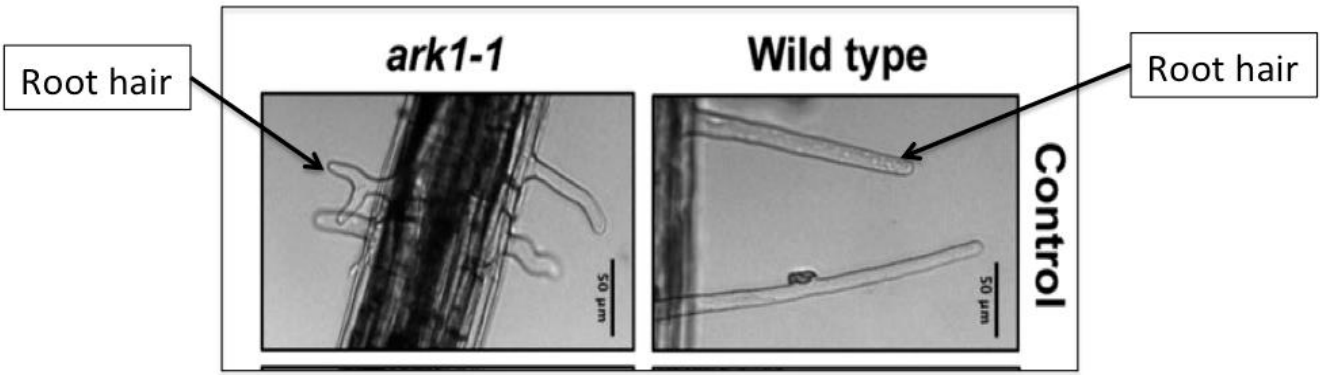
The graph on the right shows the equilibrium distribution of actin in free subunits (i.e monomer) compared to actin in filaments, as a function of actin concentration.

a) Describe the change in monomer and filament mass as actin concentration increases.
b) Draw a line on this graph to indicate clearly where the critical concentration of actin can be found.

Problem 6.3.2

(tags: #actin, #brightfield microscopy, #mutant analysis, #dynamic instability, #plants)

Root hairs are tubular extensions of epidermal cells that protrude out from plant roots. They grow through a highly polarized process called tip growth. Microtubule dynamics play an important role in this process. ARMADILLO-REPEATKINESIN1 (ARK1) has been shown to increase microtubule catastrophe events. Plants with mutations in ARK1 have been observed to have reduced catastrophe frequency and slower growth velocities in their root hair microtubules (Source: Eng & Wasteneys, 2014, Plant Cell 26: 3372-3386)



a) The micrographs show root hairs of wild-type and ARK1 mutant cells (called *ark1-1*). Describe the change in root hair phenotype in ark1-1 plants, compared to Wild type plants.

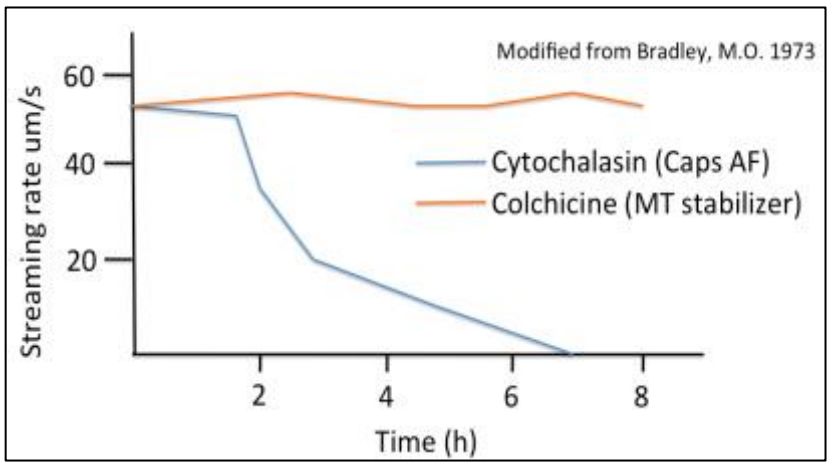
b) What can you conclude about the importance of ARK1 in the growth of root hairs?

c) Why might catastrophe be important for rapid microtubule growth?

Problem 6.3.3

(tags: #actin, #drug treatments, #plants)

Cytoplasmic streaming in plant cells is the result of the directed movement of organelles using the cytoskeleton. In the following experiment, 2 drugs were tested in *Nitella* sp., a type of alga, to see if they affected the movement of organelles in the cell.



a) Describe the change in streaming rate over time for plant cells treated with cytochalasin and colchicine.

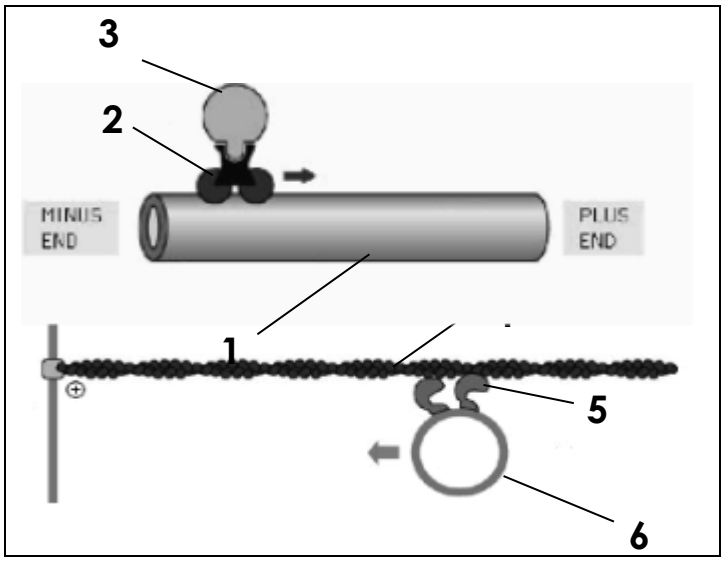
b) What conclusions can you draw about cytoplasmic streaming based on this data?

c) Which motor protein(s) is/ are most likely to be used for moving organelles around the cell in this species?

Problem 6.3.4

(tags: #actin, #motor proteins, #fluorescence microscopy)

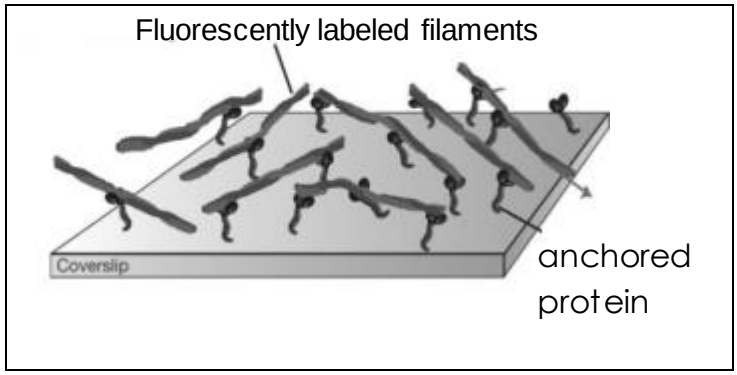
Name the structures that are labeled 1-6 in the two diagrams inside the box.



In the image below, an experiment is illustrated where researchers have immobilized proteins, such as those identified by #5 in the box, by linking their tail domain onto a coverslip. They then added fluorescently tagged filaments, such as those identified by #4.

a) What do you expect would happen when ATP was added to the experiment?

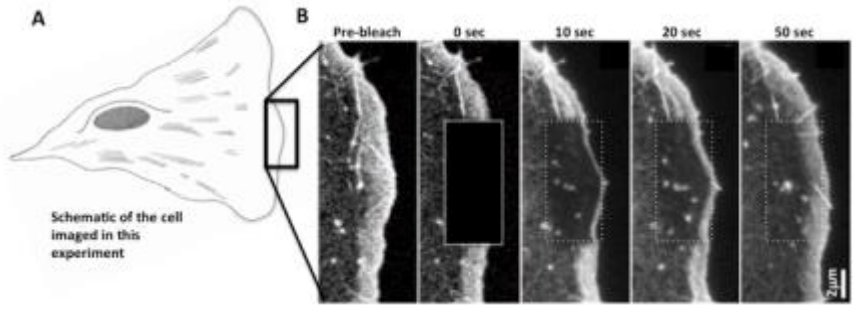
b) What if there was no ATP added to the experiment?



Problem 6.3.5

(tags: #actin, #locomotion, #FRAP, #fluorescence microscopy, #animals)

To study the dynamics of actin polymerization in crawling cells, cells expressing fluorescently labeled G-actin were examined using FRAP (fluorescence recovery after photobleaching) experiment. Shown in the figures below are the results of the experiment for a cell that was photobleached at its lamellipodium near the leading edge of the cell at time zero. The boxed region in Panel A indicates the area of the cell that was imaged, and the data is shown in Panel B.



a) Describe the fluorescence pattern seen pre-bleach, and what that fluorescence looks like it recovers from the bleach.
b) What does this tell us about actin microfilaments in lamellipodia? Explain.
c) Predict what would happen if the FRAP experiment was performed in the presence of Cytochalasin B (a drug that caps the plus end of actin filaments). Would the cell continue to migrate?
d) Monomeric actin isolated from these cells was tested for polymerization in a test tube and its critical concentration was found to be 0.1 μM for the plus end. However, when the concentration of actin monomers in the cytosol of these cells was measured, it was found to be 100 μM. This is 1000 times higher than the critical concentration of actin monomers in the test tube. What keeps these monomers from polymerizing totally into filaments in these cells?

Problem 6.3.6

(tags: #actin, #brightfield microscopy, #drug treatments, #locomotion, #experimental controls, #prediction, #animals)

When an animal cell line is grown in culture, as seen in the image to the right, cells actively crawl around in the Petri dish. You suspect that either microtubules or actin microfilaments are involved in cell movement.

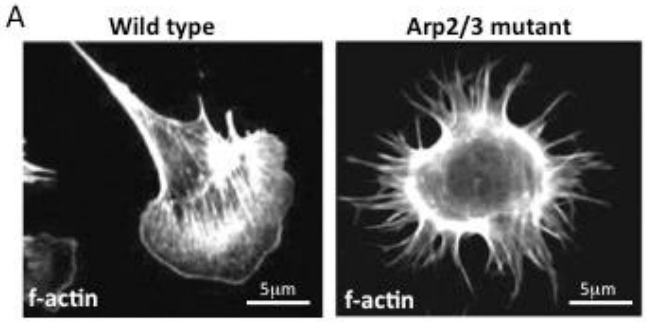
Note: Read parts A, B, and C before starting to answer part A.

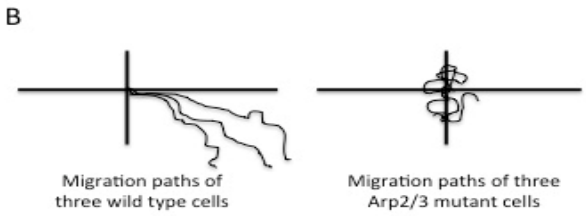
a) Describe the drug treatments you would carry out in order to test conclusively which cytoskeletal element is responsible for the movement of cells across the petri dish and briefly explain why.
b) Describe the control experiment for your study.
c) Predict the results that you would expect to see in your experiments in A and B. Explain your reasoning.

Problem 6.3.7

(tags: #actin, #fluorescence microscopy, #mutant analysis, #locomotion, #drug treatments, #prediction, #animals)

Arp2/3 is an actin binding protein complex that nucleates new actin filaments. Suraneni *et al*. (2012) studied the role of Arp2/3 in migration of fibroblast cells by comparing the labeling pattern of f-actin (panel A) and the migration paths (panel B) of wild type cells versus cells with a loss of function mutation of the arp2/3 complex.





Note: the x and y directions are indicated by the cross. The movement of the cells was measured from the point of intersection of the x- & y-axes.

a) Describe the data in Panel A. How does the Arp2/3 mutation affect the cell?

b) Describe the data in Panel B. How does the Arp2/3 mutation affect cell migration?

c) Why do Arp2/3 mutants show changes in both cell shape and migration? Explain your reasoning.

d) Predict the effects on f-actin dynamics and cell motility if the wild type cells were treated with phalloidin (drug that binds and stabilizes actin filaments) for 10 minutes. . Why?

Unit 7: Cell Cycle and Mitosis

Content Review Questions

- This section is designed to help you review the material. Answers can be found more or less easily in your notes, on Connect, in the textbook or on the Web. While this background content knowledge is needed and will help you on the exam, these review questions are not considered 'exam-style'.
- 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.
- There are no answer keys to questions in this section. We suggest using these questions as a way to review & discuss the material with your peers, rather than writing out answers for each one.

Topic 7.1 – Cell Cycle & Checkpoints

a) Make a list of the 4 phase of the cell cycle, and describe in detail what has to happen in each phase before it is complete. Be as specific as you can. (1)
b) For each of the stages, make a list of experimental techniques that could be used to identify cells that are in each of the 4 stages. Explain your rationale for your choice in each case. (2)
c) Explain the concept of checkpoint control of cell cycle and give examples of what types of conditions should be met before S and M phases proceed. (1) Explain why those conditions need to be met before the cell moves to the next phase. (3)

Topic 7.2 – CDK-Cylin Regulation

a) Describe the sequence of events leading to CDK activation and deactivation. (1)
b) Define negative and positive feedback. How are they thought to play a role in cell cycle control? (1)
c) Discuss the role of CDK-cyclin complexes in regulation of the cell cycle. How do they contribute to the cell 'knowing' the conditions are right for the next stage of the cell cycle? (2)
d) Compare and contrast the roles of S-CDK and M-CDK. Make a list of the different examples of proteins (listed in the textbook or on Connect) that they are thought to interact with, and their effects. (2)
e) Explain the role of phosphorylation in regulation (1). Explain how phosphorylation can affect protein folding, and how that might result in changes in protein activity. (3)

Topic 7.3 – Mitosis and Cell Division

a) Describe the role of dynamic instability in the formation & maintenance of the mitotic spindle.(2)
b) Illustrate with labeled diagrams the relationship between chromatids and chromosomes at each of the stages of the cell cycle. (2)
c) Explain how the stages of mitosis are ultimately controlled by the activation and deactivation of CDKs. (3)

Practice Problems

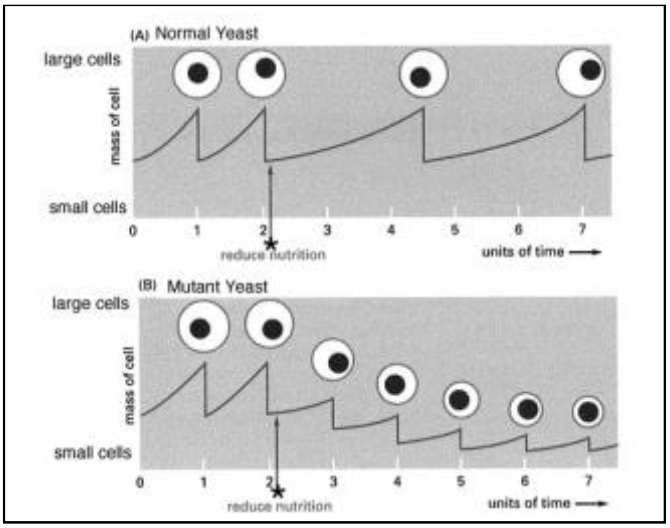
- The focus on the exam will be to assess your ability to interpret data, and use it to draw conclusions about cellular function. This is a conceptual task, which requires critical thinking skills in addition to the knowledge of the material. But practice will help you get good at it.
- Most of these questions come from old exams. Some of them are designed to make you think, rather than to practice exam-writing.
- We will cover many of these questions in class and/or tutorial. We encourage you to practice writing out your answers for these questions, as it will help you improve your clarity in writing for the exam.
- 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 7.1 – Cell Cycle & Checkpoints

Problem 7.1.1 (Walkthrough Available)

(tags: #cell cycle, #drug treatments, #protein regulation, #mutant analysis, #yeast)

The diagrams on the left show the relationship between growth rate, cell size, and cell cycle control in yeast. The "mass of cell" refers to the size of an individual cell followed over repeated rounds of the cell cycle, as shown in the drawing of the yeast cell.
In the top panel, normal yeast are subject to reduced nutrition (*). In the bottom panel, a mutant is subject to reduced nutrition (*).



a) Describe how decreasing the nutrients available changes the cell cycle and cell size in the normal yeast.
b) Describe how decreasing the nutrients available changes the cell cycle and cell size in the mutant yeast ?
c) The mutation was found to be in the gene encoding an **inhibitor** protein that binds to G1/S cyclin-dependent kinase and blocks its activating phosphorylation site. Explain how loss of function of the inhibitor could produce these results. First briefly outline the mechanism of cell cycle control responsible for entry into S in normal yeast, and then indicate why the mutation would cause the observed effect.

Problem 7.1.2

(tags: #cell cycle)

The shortest cell cycles of all species occur in the early phases of animal embryonic development. These divisions take place without any significant increase in the weight of the embryo (see the video showing Frog embryonic development on the 'Visualizing the Cell' page for an example of this). How can this be? Which phase of the cell cycle would you expect to be the most reduced (and why)?

Problem 7.1.3

(tags: #cell cycle, #protein regulation)

Indicate in which stage(s) of the cell cycle each of the following statements occur(s): the G1 phase, the S phase, the G2 phase, or the M phase.

a) The amount of nuclear DNA in the cell doubles.
b) The nuclear envelope breaks into fragments.
c) Sister chromatids separate from each other.
d) Cells that will never divide again are likely to be arrested here.
e) Chromosomes are present as diffuse, extended chromatin.
f) Cyclin-dependent kinase phosphorylates DNA replication machinery.
g) Mitotic cyclin is at its lowest level.
h) Cdk proteins are present in the cell.
i) S-Cyclin is at its highest level..

Problem 7.1.4

(tags: #cell cycle, #yeast)

Several factors are monitored by yeast cells and used to make the decision of whether or not to pass the G1/S checkpoint and commit to cell division. Presence or absence of Mating Factor is one of the factors monitored. Mating Factor is a peptide hormone that is produced by cells of one mating type to cause cells of the complementary type to prepare to mate. If the mating factor is present, cells will not pass the G1/S checkpoint and instead they will immediately halt the cell cycle. If they can find a mate they enter the mating pathway. In cultures of a single mating type, however, the cell cycle is arrested as long as the mating factor is present.

Experiment: You prepare a sample of mating factor from a culture of one mating type of yeast, and then add mating factor to an asynchronous culture of cells of complementary mating type. This culture contains cells at all stages of the cell cycle. Because there is only one mating type present, the cells can't enter the mating pathway.

Consider three cells:

- one in early G1,
- one in early S,
- and one in G2 stage.

Make cell cycle diagrams showing what happens to each of these cells after mating factor is added. Indicate the stages of the cell cycle through which the cells pass.

a) Do they all eventually stop (cell cycle arrest)?
b) Do they all stop at the same stage?
c) Do they all stop at the same time?
d) What will happen if the mating factor is removed from a population of cells after they have been arrested?

Problem 7.1.5

(tags: #cell cycle, #yeast)

Background: Yeast cells divide through a budding process. The cell cycle is divided into two parts, the G1 period during which the cell has no growing bud, and the remainder of the cell cycle during which DNA replication and mitosis occurs (See the external link above for more information). This second period of the cell cycle is initiated when the bud begins to form. During the bud-less portion of the cell cycle, the cell increases in size. After bud initiation has occurred, all growth takes place in the bud and the mother cell does not grow.

a) How is this different than the typical eukaryotic cell cycle?

In yeast cells, DNA replication is initiated (noted by IDS in the figure below) at the same time that the new bud begins to form. Both of these events occur immediately after has passed the G1/S checkpoint. Within a few minutes after passing the G1/S checkpoint, the cells become committed to division and must pass through S, G2 and M (Figure A, below). If a random culture of yeast cells, containing cells at all stages of the cell cycle is transferred to medium with very little food, the rate of cell growth decreases greatly. Many cells eventually reach division; some of the daughter cells produced are very small, while others are larger (Figure B, below). Note that numbers beside the diagrams indicate the mass of the cells in arbitrary units.

Figure legend:
Part A: The pattern of bud formation and cell growth in normal well-fed cells.
Part B: A mixture of newly divided cells from an asynchronous culture that had been grown for some time under poor nutrient conditions.

b) Why are the cells in B more variable in size than the ones in A? Why do they reach division at different rates?

c) Assume that the cells in figure B above are all newly divided cells in G1 and have just been returned to rich medium. Make a diagram showing the relative duration of the G1 (unbudded) and budded (S + M) portions of the cell cycle for each of the cells. Assume that there is a constant growth rate of 70 units of cell mass per hour. What assumption about cell cycle checkpoints do you need to make to do the problem? If you need help understanding this Problem, there are some animations in the Resources page of Connect to help you

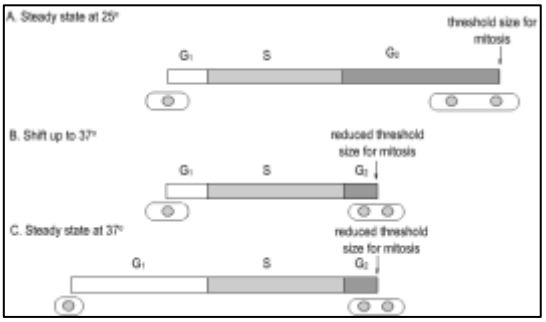
Problem 7.1.6

(tags: #cell cycle, #temperature sensitive mutants, #mutant analysis, #yeast)

Note that fission yeast have a different mechanism of division than budding yeast. Like the name says, these yeast divide by fission, much like bacteria do.

Fission yeast are a very good organism in which to study cell cycle regulation. These eukaryotic cells grow, but only in one dimension (length) and divide in half after mitosis by a process of septation, rather like the mechanisom observed in a bacterium. There is a class of mutations affecting the fission yeast cell cycle that result in abnormally small cell size at division. These mutants are called '*wee*' mutants because the cells are small and the mutants were produced and studied at the University of Edinburgh in Scotland.

One of these mutations (*wee1ts*) is temperature sensitive. That is, at 25°C the wee1ts protein has normal activity and the cell divide at normal size. At 37°C the protein has no biological activity and the cells divide at about half of the normal size.



In the normal cell cycle of fission yeast, the G_1 period is shorter and the G_2 period is longer, relative to each other. This cycle looks the same as the *wee1ts* conditional temperature-sensitive mutant when it is grown at 25°C, which is shown in Part A of the figure below. The daughter cells are the same size as the mother cell was when it was freshly divided.

If cells are shifted to 37°C, they divide at a smaller size with a shortened G_2 period and shortened cell cycle (Part B of figure). If cells continue grow at 37°C, the restrictive temperature, they undergo a second cell cycle of normal length, but with longer G_1 and shorter G_2 than at 25°C (Part C of figure).

a) Can you explain what is happening here?

b) Based on your knowledge of cell cycle regulation, how can you account for the changes in the timing of cell cycle events?

Problem 7.1.7 (Covered in Tutorial)

(tags: #drug treatments, #mutant analysis, #temperature sensitive mutants, #cell cycle)

Temperature sensitive (ts) mutations have been an important tool for understanding cell cycle regulation. These mutants grow and divide normally at one temperature (the permissive temperature) and are blocked at some particular cell cycle stage at a higher temperature (the restrictive temperature).

You are analyzing three new ts mutants (generic, unspecified cells) and you are trying to find out where in the cell cycle the mutants are blocked. Your experimental design is as follows:

- For each mutant you apply one treatment for one cell cycle, then apply a second treatment for a cell cycle.
- After the second treatment you check to see if cell division has occurred.
- You then reverse the order of the treatments, and repeat the experiment.

The two treatments used in this experiment are:

- **Chemical DNA Synthesis Block**
  Inhibits DNA synthesis for one full cell cycle.
  (note: the chemical inhibitor is reversible, so after the treatment DNA synthesis can begin)
- **Restrictive Temperature (genetic block)**
  Blocks cell cycle at an unknown stage, which is different for the different mutants.

	Mutant #	First Treatment	Second Treatment	Result
Experiment 1	**M1**	Chemical DNA synthesis block	Restrictive temperature (genetic block)	No cell division observed
	M2	Chemical DNA synthesis block	Restrictive temperature (genetic block)	No cell division observed
	M3	Chemical DNA synthesis block	Restrictive temperature (genetic block)	Cells undergo mitosis
Experiment 2	**M1**	Restrictive temperature (genetic block)	Chemical DNA synthesis block	No cell division observed
	M2	Restrictive temperature (genetic block)	Chemical DNA synthesis block	Cells undergo mitosis
	M3	Restrictive temperature (genetic block)	Chemical DNA synthesis block	No cell division observed

Based on this data, can you figure out where the genetic block is in each mutant? Explain how each experiment helped you come to these conclusions.

Mutant #	Stage affected by mutation:
	G1/S checkpoint
	DNA replication
	G2/M checkpoint

Topic 7.2 – CDK-Cylin Regulation

Problem 7.2.1

(tags: #mutant analysis, #cell cycle)

What do you suppose is the phenotype of cells that bear the following mutations? Explain.

a) They cannot degrade M-cyclin.
b) They lack an enzyme that adds the inhibiting phosphate to the M-cyclin-Cdk complex.
c) They have a mutation in p53, the protein that checks for DNA damage prior to S-phase.

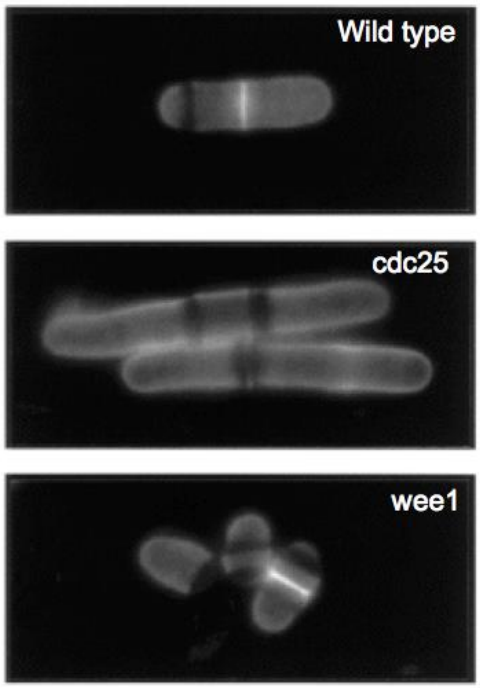
Problem 7.2.2

(tags: #fluorescence microscopy, #mutant analysis, #temperature sensitive mutant, #mitosis, #cell cycle, #yeast)

One of the most powerful genetic tools a research scientist has is the capacity to produce mutant genes. Prior to the development of fast and easy genome sequencing, scientists had to rely more heavily on phenotypic studies, but the premise for this type of experiment remains unchanged since its first usage in the 20th century. The two main types of mutational changes that are most useful to scientists are:

- Loss-of-function (lof) mutants, in which the amount of wild-type gene product is severely reduced (sometimes, but not always, to the point of being completely eliminated),
- Gain-of-function (gof) mutations, in which the cell loses its ability to control expression of that gene, so that the gene is 'always on' resulting in much higher concentrations of gene product that the cell would normally have.

In the image on the right, several fission yeast can be seen just prior to their entry into M-phase. The division plane can be seen in the centre of each of the cells, thus producing daughter cells of equal size. The wild type is shown in the top panel, and 2 different cell-cycle regulatory gene mutants are shown in the panels below.



a) Describe what happens in both the wee1 and cdc25 mutations shown in the images.

b) How do you think that each of these genes normally acts in the control of division?

c) What do you think would be the effect of:

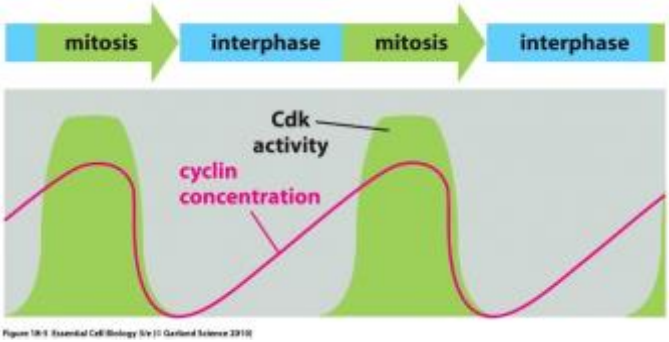
- Increasing dosage of wee+ wild type allele to three copies vs one?
- Increasing dosage of cdc25+ wild type allele to three copies from one?

d) The proteins encoded by the genes used in these studies encode important cell cycle regulatory proteins. What do these proteins do? Does this knowledge cast any further light on the implications of these gene dosage studies for cell cycle regulation? Explain.

Problem 7.2.3

(tags: #cyclin, #CDK, #cell cycle, #mitosis)

This figure from your textbook shows the rise of cyclin concentration and the rise of M-Cdk activity as they progress through the cell cycle.



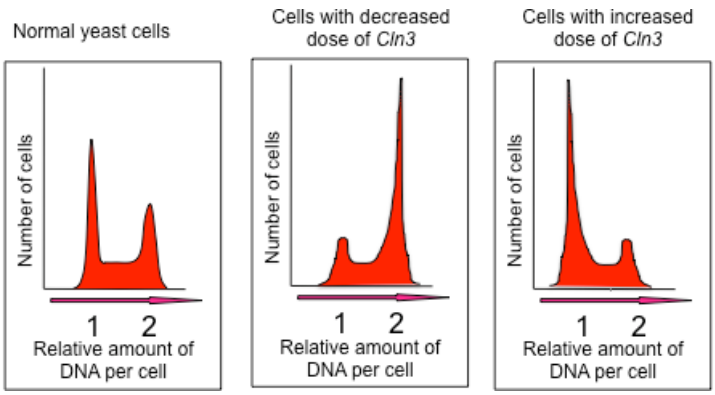
a) The graph shows that cyclin concentrations rise slowly and steadily, whereas M-CDK activity increases quite suddenly. How do you think this difference arises?

b) One of the functions of M-CDK is to cause a precipitous drop in M-cyclin concentration halfway through M-phase. Describe the consequences of this sudden decrease and suggests possible mechanisms by which it might occur.

Problem 7.2.4

(tags: #FACS, #cell cycle, #mutant analysis, #yeast)

Budding yeast contains several cyclins that act at different phases of the cell cycle. To study the role of one of these yeast cyclins (named Cln3) you performed gene dosage experiments by increasing or decreasing the Cln3 genes in the cells. To determine where in the cell cycle Cln3 acts you exposed the cells to a fluorescent dye that binds DNA and then sorted the labeled cells in a flow cytometer. Below are the data from your experiments.



a) Describe the change in DNA amount in normal yeast cells, and in yeast cell dosed with less, or more, *Cln3*.

b) The x-axes differentiate between 1 and 2 'relative amounts of DNA per cell'. What stage(s) of the cell cycle are the cells at when the have these different amounts of DNA?

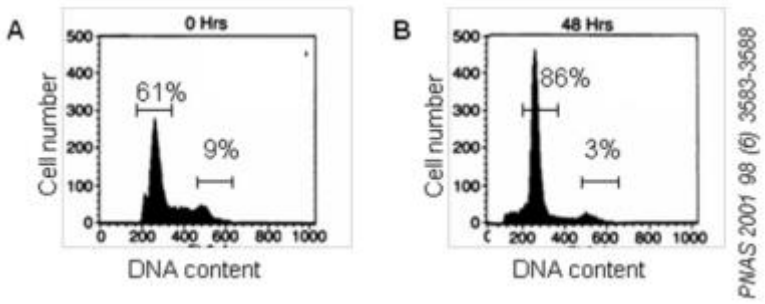
c) Based on the data what conclusion can you make about where Cln3 acts in the cell cycle?

d) Would you expect the daughter cells to be smaller than normal, the same size, or larger than normal after one cell cycle. Provide a rationale for your decision.

Problem 7.2.5

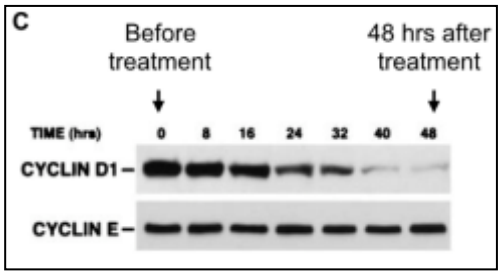
(tags: #SDS-PAGE, #FACS, #mitosis, #drug treatments, #animals)

In this experiment by Pervin et al. (2001), rapidly cycling (dividing) cells from a human breast cancer cell line were treated with the drug DETA-NONOate. DNA content was measured by FACS before treatment (Panel A) and 48 hours after treatment (Panel B). The percentages refer to the number of cells in each peak.



a) Describe the change in DNA content before and after treatment with DETA-NONOate. What does this tell you about the progression of the cell cycle in the cells in panel A and B?
b) Based on this data, what effect does DETA-NONOate have on the cell cycle?

Pervin et al. (2001) also used SDS-PAGE to examine the impact of DETA-NONOate on levels of cyclin D1 and cyclin E in these cells. Results are shown here in panel C. **Note that untreated cells at 48 hrs would continue to resemble the results seen at 0 hrs.**

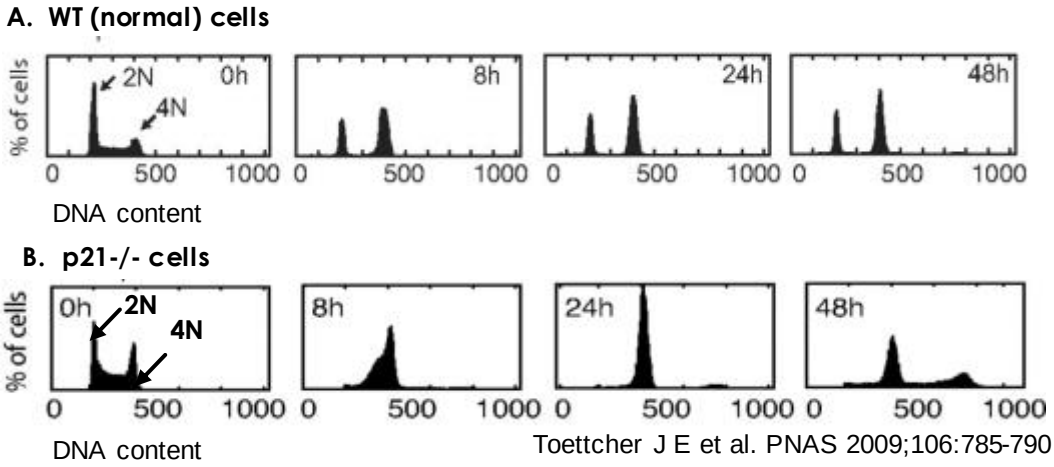


c) Based on the results of these two experiments, propose a mechanism for how DETA-NONOate affects cell cycle.

Problem 7.2.6

(tags: #FACS, #DNA damage, #mutant analysis, #animals)

Toettcher et al. (2009) examined the impact that gamma-irradiation and DNA damage had on cell cycle arrest in wild-type and p21-/- mutant cells (cells that lack the protein p21). They ran an experiment where cells were exposed to gamma-irradiation and then measured the DNA content by FACS at the indicated time-points.



Toettcher J E et al. PNAS 2009;106:785-790

a) Describe the data in Panel A. What happens in Wild-type cells when the DNA is damaged by gamma irradiation?
b) What happens when p21 absent (Panel B) and the DNA is damaged?
c) What can you conclude about the role of p21 in cell cycle?

Problem 7.2.7

(tags: #mutant analysis, #temperature sensitive mutations, #cell cycle, #FACS, #yeast)

You generated a yeast strain with a temperature-sensitive mutated form of M-cyclin that is unable to fold stably at a non-permissive temperature (37°C). To assess its role in the cell cycle, you grow these mutant cells in a test tube at the permissive temperature (25°C) then shift them to 37°C.

a) Explain what happens to M-cyclin concentration as the cell progresses thorough interphase at the permissive temperature. How does this compare at the non-permissive temperature?
b) What effect would this mutation have on the regulation of cell cycle at the non-permissive temperature?

You fluorescently labelled the DNA of your mutant yeast cells and used fluorescence activated cell sorting (FACS) to analyze the effect of this mutation on the cell cycle. Note that these cells complete a cell cycle in 90 minutes where interphase lasts for approximately 70 minutes.

1. In the first part of your experiment, you incubate the yeast cells at the non-permissive temperature, 37°C, for 2 hours. At this point you take a FACS reading.
2. You follow the first incubation with a second incubation of 30 minutes at the permissive temperature. You take a second FACS reading here.

c) Draw the expected FACS readouts from your experiment and label the graphs with the relevant phase of cell cycle expected. Explain in words the results that you drew on your graphs.

Topic 7.3 – Mitosis and Cell Division

Problem 7.3.1 (Walkthrough Available)

(tags: #essay outline, #microtubules, #actin, #mitosis, #cell cycle)

Here is a sample argument outline question for you to practice with. For this Problem, 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.

> *The process of mitosis depends on interplay between the cytoskeleton and chromosomes while the regulation of mitosis entry and exit depends on cytoplasmic proteins. Write an outline that argues the importance of protein activation/deactivation for mitosis (both the process, and the regulation). Provide an overall thesis statement and three supporting arguments (with evidence) to support your arguments.*

Problem 7.3.2

(tags: #fluorescence microscopy, #mitosis)

a) Where would you expect a fluorescent tag for telomeric DNA repeats to be localized in an interphase cell? Why?
b) Where do you think fluorescent-tagged tubulin would localize in living cells during each of the stages of mitosis? Explain your reasoning.
c) Where do you think fluorescent-labeled nucleolin, a protein that is involved in structural organization of the nucleolus, would be localized during each of the stages of mitosis? Explain your reasoning.
d) What would happen to the localization of nucleolin in the anaphase cells as they completed mitosis and entered interphase again? Why?

Problem 7.3.3

(tags: #drug treatments, #actin, #microtubules, #mitosis, #prediction)

Predict the effect you would expect to see on mitosis for the following cytoskeletal drugs, and explain your rationale for your prediction. Make sure to address: what stage would be disrupted (if any), the nature of the disruption, and the result. (note: You may need to look some of these drugs up to find out what they do).

a) Colchcine
b) Taxol
c) Nocodazole
d) Latrunculin
e) Cytochalasin B

Problem 7.3.4

(tags: #drug treatments, #actin, #microtubules, #mitosis, #prediction, #plants, #animals)

For the drugs listed above, would the effects that you predicted be the same in a plant and an animal cell? Why or why not?

Example Problem Walkthroughs

Introduction to the Problem Walkthroughs

What is an example problem walkthrough?

We have chosen a selection of practice problems from the problem sets that highlight key skills or types of problems that are especially important for Biology 200. For these problems, we have written **Problem Walkthroughs**, designed not only to show you what the answer to the question is, but to help you understand why its correct, and to better understand what a 'good' answer contains. Each walkthrough includes an **Example Answer**, as well as **Instructor Commentary** explaining the solution and how it was reached.

How can I use the problem walkthroughs to help me study?

1. **When Stuck on Problems Without Walkthroughs**
   The idea behind the problem walkthroughs, is that they will help you with other problems that do not have a walkthrough. If you get stuck on a problem, look for matching blue *(tags:)* in the problem walkthroughs, and use these to help get un-stuck.
2. **Find Similar Types of Problems to Practice On**
   After reading a walkthrough, you can find related problems to test your skills, by looking up the relevant *(tags:)* in the index. Attempting these related problems on your own is an important step, since it will help prepare you for what you will face in the exams.
3. **Get an Idea of How to Answer Problems on the Exams**
   The example student answers in the walkthroughs can help you get an idea for what kind of answers we expect on the midterm and final exam. This may help you write similar types of answers when doing the practice problems, and when writing your exams.
4. **Get Tips and Tricks for Working Through Different Genres of Question**
   There are several common types of questions that you can expect to see in Biology 200, and these show up several times in the example problem walkthroughs. For example, skills such as describing data given, and drawing conclusions from that data, are likely to come up during exams. The instructor commentary in the walkthroughs often provide tips and tricks for answering these types of questions, which you may find valuable.

Where to find more help?

We understand that the practice problems can be challenging, and the problem walkthroughs might not provide as much help as you need. If you need additional help, you can form study groups, post on the discussion board, or come to office hours.

Unit 1: Eukaryotic Cells and Microscopy

Identifying Organelles and Cellular Structures Using Microscopy

Problem 1.2.1

(tags: #TEM, #SEM, #brightfield microscopy, #fluorescence microscopy, #organelle identification, #identify microscopy type, #plants, #animals)

Example Answer: Orange Text
Instructor Comments: Blue Text

Classify each of the images shown into the table below, based on the type of microscopy and the organelles that you can SEE. Be aware that each image may fit into more than one category and not all categories may be present. Marks will be deducted for wrong answers.

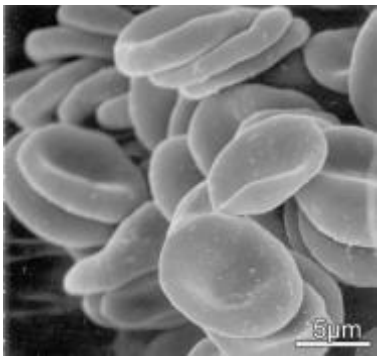


IMAGE 1
Type of Microscopy: Scanning Electron Microscopy
Explanation: In this image we are seeing the surface of many 3D structures, suggesting SEM.
Organelles: Plasma Membrane
Explanation: Eukaryotic cells are usually range from 5-50µm. The scale bar suggests that these structures are ~15µm in diameter, so they are much too big to be organelles, and are more likely to be cells. This would mean we are looking at the 3D structure of the outer leaflet of the plasma membrane of these cells.

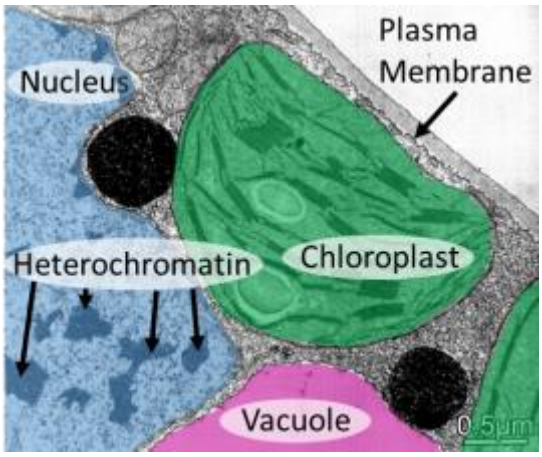
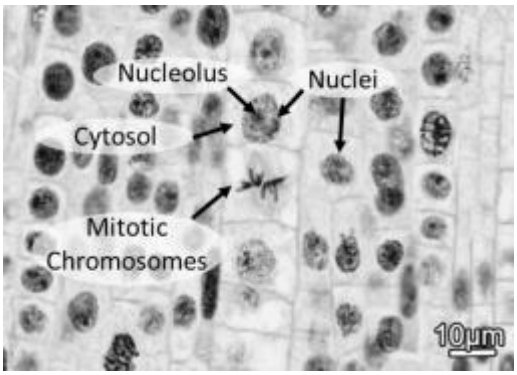


IMAGE 2
Type of Microscopy: Transmission Electron Microscopy
Explanation: The scale bar tells you the magnification is quite large, and the resolution of the image is also very good. It also looks like you are seeing cross-sectioned material. All this suggests this is TEM.
Organelles:
Chloroplasts (**easiest**: Chloroplasts are around 4µm long. We can also see the characteristic thylakoid membranes stacked in grana inside.)
Cytosol (**easiest**: Surrounding the chloroplast and other organelles)
Nucleus, Heterochromatin (**harder**: On the left side of this image you can see a structure with the double membrane characteristic of the nucleus. Note the difference in texture compared to the cytosol. We can only see part of this structure, but it is of a similar size to the cell nucleus which usually around 10µm in diameter. There are also darker and lighter regions inside, as we would expect for a nucleus containing heterchromatin and euchromatin.)
Plasma Membrane (**hardest**: the light area at the top right is probably the outside of the cell, making the membrane in between the plasma membrane. You might also notice a grey layer outside the plasma membrane, which is the plant cell wall.)

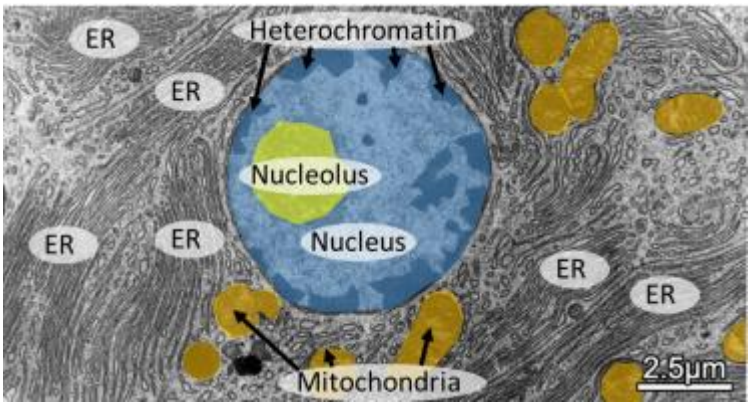
Vacuole (**hardest**: the light areas at the bottom of the image is likely the large plant vacuole)
Other Structures (The two dark circles, and the oval structures above the chloroplast, are hard to identify even for experts. Some of them may be mitochondria, but more information about the samples, and more experiments, may be required to say for sure.)

IMAGE 3



Type of Microscopy: Brightfield Light Microscopy
Explanation: The scale bar indicates a low-magnification image, and the lack of resolution suggests this is brightfield microscopy, rather than TEM.
Organelles:
Explanation: The scale bar suggests the repeating structures arranged in columns are quite large (10-15µm across), and therefore probably cells (see explanation for image 1).
Nucleus, Nucleolus (The dark circles inside each cell are around 10µm across, which is the right size for a nucleus. They also contain an even darker spot, which is the nucleolus)
Mitotic Chromosome (Some of the cells are undergoing cell division, as we can see chromosomes at different stages of mitosis. As we would expect, the circular nuclei are not present in these dividing cells.)
Cytosol (There is not enough resolution to see the smaller organelles that would be present in these cells, however you know that the cytosol is filling these cells and surrounding the nucleus.)

IMAGE 4



Type of Microscopy: Transmission Electron Microscopy
Explanation: Similar to image 2, you are looking at an image at very high magnification and very high resolution, and it looks like a cross-section of a cell, suggesting TEM.
Organelles:
Nucleus, Nucleolus, Heterochromatin (The circular structure in the center is around 10µm in diameter and is surrounded by a double membrane, just as we would expect the nucleus to be. We can also see the darker nucleolus inside the nucleus. There are other patches of dark material, especially around the margins of the nucleus, where we expect heterochromatin to be more abundant.)
Mitochondria (TEM mitochondria tend to be darker than many organelles, like the dark oval structures seen here. These structures are also the right size, as mitochondria are usually 1-2µm long, and 0.5µm in diameter. We can also see the inner cristae membranes. The variation in shape of these mitochondria is due to their different orientations relative to the plane of section.)
Endoplasmic Reticulum (This cell contains many layers of long, thin threads of membrane with very lightly stained contents. This is typical of ER.)
Cytosol (The cytosol surrounds all of these other organelles.)

TUTORIAL: Understanding the Plane of Section in Microscopy Images

Problem 1.2.5 (Covered in Tutorial, Answer Online)

(tags: #TEM, #nucleus, #plane of section)

TUTORIAL: How to Select the Appropriate Type of Microscopy to Answer Different Scientific Questions

Problem 1.2.6 (Covered in Tutorial, Answer Online)

(tags: #identify microscopy type)

TUTORIAL: Using Micrographs to Identify the Type of Microscopy and the Organelles Imaged

Problem 1.2.7 (Covered in Tutorial, Answer Online)

(tags: #identify microscopy type, #organelle identification, #plants, #animals)

Unit 2: Biological Membranes

TUTORIAL: Essay Outline (Unit 2: Membrane Fluidity)

Problem 2.1.3 (Covered in Tutorial, Answer Online)

(tags: #essay outline, #membrane fluidity, #animals)

Using Fluorescence Recovery after Photobleaching (FRAP) to Investigate Protein Mobility

Problem 2.1.5

(tags: #transmembrane domains, #protein structure, #FRAP, #animals)

Example Answer: Orange Text
Instructor Comments: Blue Text

A. The transmembrane protein glycophorin spans the lipid bilayer of a red blood cell with a single α-helix. Which of the 3 amino acid sequences below is the most likely to be the membrane-spanning domain of this protein? Explain your reasoning.

1. I T **L** I Y F G **V** M A G V I G T **I** L L I S
2. I T **T** I Y F G **S** M A G V I G T **Y** L L I S
3. I T **E** I Y F G **R** M A G V I G T **D** L L I S

Answer: *Sequence 1 is the most likely.*

Explanation: Should address both why Sequence 1 fits the criteria of the question, **AND** why the other sequences don't. For example:

- *Sequence 1 is composed predominantly of hydrophobic amino acids, which is required to span the hydrophobic region of the membrane.*
- *Sequence 2 contains 3 more polar amino acids, making it less favorable than 1.*
- *Sequence 3 contains three charged amino acids, whose presence in the hydrophobic lipid bilayer would be energetically unfavorable.*

B. Why is it that membrane-spanning domains of proteins are almost always α-helices or β-barrels, but never disordered chains?

To fully answer this question, you must explain why α-helices and β-barrels are suitable for spanning a membrane, AND why disordered chains are not. For example:

- *The lipid bilayer is a hydrophobic environment.*
- *The amino acid backbone is always hydrophilic.*
- *α-helices and β-barrels have secondary structure that prevents the hydrophilic amino-acid backbone from interacting with the hydrophobic environment inside the bilayer.*
- *However, the hydrophilic backbone is exposed in a disordered chain. It is therefore energetically unfavourable for a disordered chain to span a membrane.*

C. You want to run an experiment to see whether glycophorin is anchored to another protein inside the cell. You decide that a FRAP experiment is the best way to address this question. Why?
A correct answer must describe the principles of FRAP, and why this technique is appropriate in this case. For example:

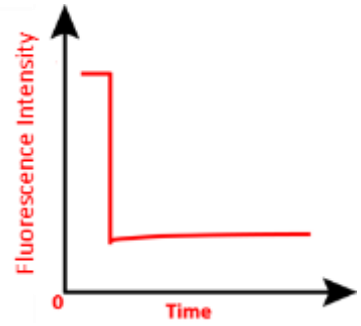
> *In FRAP, a protein or lipid in question is tagged with a fluorescent probe. A single area in the cell is then bleached. The more quickly fluorescence returns to the bleached area, the higher the lateral mobility of the fluorescent probe. If glycophorin is anchored to another protein in the cell, we would expect little to no recovery of fluorescently-tagged glycophorin after bleaching.*

D. Your results indicate that Glycophorin is, in fact, anchored. Draw and label the graph of your results from the FRAP experiment for Glycophorin. If glycophorin were not anchored, how would the graph you drew be different?

For full marks, graphs must be fully labelled. You may either draw a single graph and then describe how it would be different, or show the difference on the graph you drew.

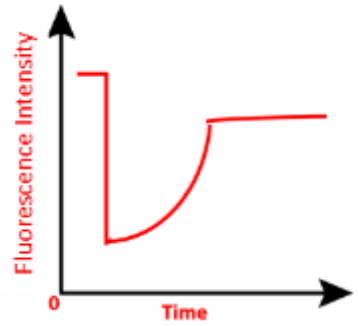
Note: Its always worthwhile to write a quick explanation, to ensure the marker understands your drawing.

FRAP Graph of Anchored Glycophorin



Instructor Explanation:
Step 1: Fluorescence intensity starts high.
Step 2: Fluorescence intensity drops very quickly as the fluorescence is 'bleached'.
Step 3: Very little increase in fluorescence occurs, as the glycophorin is anchored and un-bleached proteins cannot move into the bleached area.

FRAP Graph of Un-Anchored Glycophorin



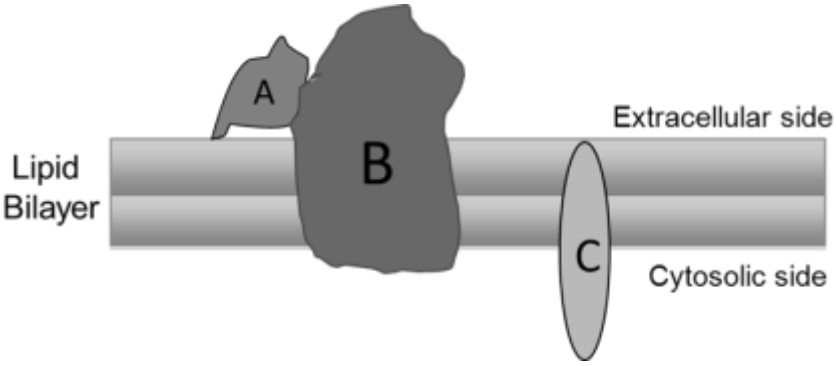
Instructor Explanation:
Step 1: no difference
Step 2: no difference
Step 3: An increase in fluorescence occurs, as un-bleached glycophorin is able to move into the bleached area, since it is not anchored.

Determining Protein Topology Using Protease Digestion and SDS-PAGE

Problem 2.3.6

(tags: #SDS-PAGE, #protein topology, #prediction)

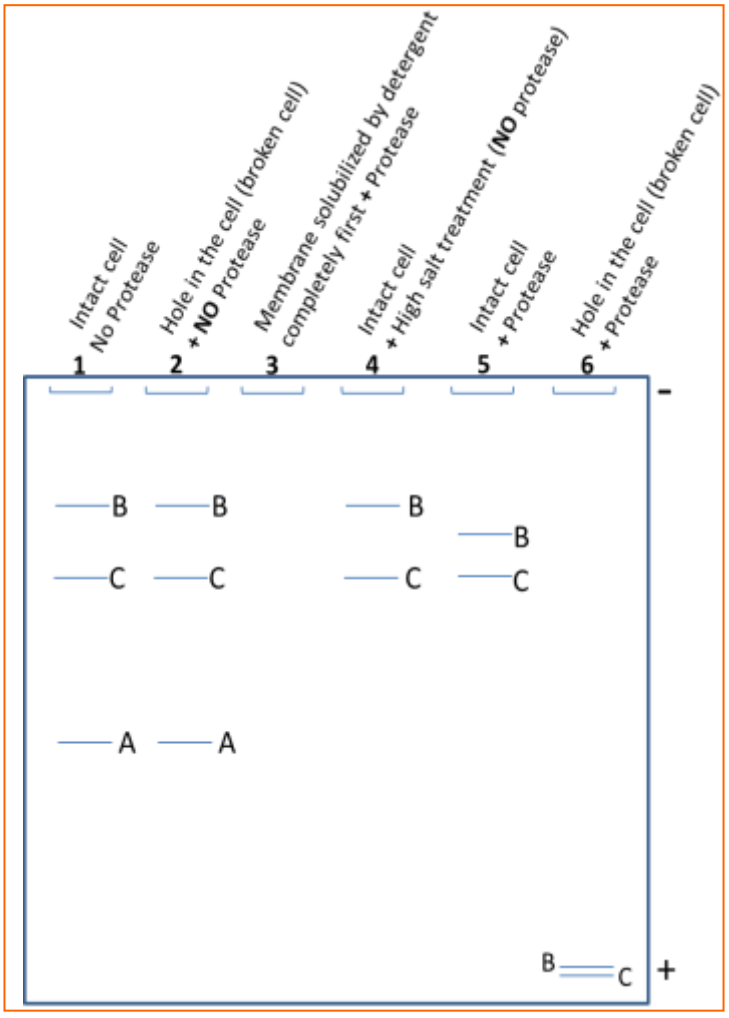
Example Answer: Orange Text
Instructor Comments: Blue Text



Shown on the right is a drawing s the arrangement of proteins on the plasma membrane of a cell.

In the following experiment, proteins A, B, and C are being extracted from membranes (pictured above) after various experimental treatments (listed below). The extracted proteins will then be separated by size using gel electrophoresis (SDS-PAGE).
Predict the results that you would expect to see in this experiment by drawing clearly labeled protein bands on the gel image below.

Note: For full marks, it is very important to clearly label each lane on the gel, and which protein is being represented by each band in the gel.



Lane 1: intact cell with **no** protease treatment
Number of Bands: If protease is not added, no part of these proteins are expected to be degraded. Therefore, we would expect three bands.
Position of Bands: This lane is a control establishing the size of proteins A, B and C when they are intact. Right now, the specific location of these proteins are not so important, but it looks like protein B is bigger than protein C, which is bigger than protein A. Therefore, protein B should be higher up the gel than protein C, which should be higher up the gel than protein A.

Lane 2: hole in cell (broken cell) with **no** protease treatment
Number of Bands: Like in Lane 1, there is no protease added. Therefore, no part of these proteins are expected to be degraded. As such, we would still expect three bands.
Position of Bands: Proteins A, B and C are not being degraded. The position of these bands should be the same as in Lane 1.

Lane 3: membrane solubilized completely by detergent first followed by protease treatment
Number of Bands: In this treatment, the membrane is no longer protecting any part of the three proteins. Therefore, all three proteins would be fully degraded by the protease treatment, and no bands would be seen.
Position of Bands: There should be no bands.

Lane 4: intact cell + treatment with a solution of high salt concentration + **no** protease treatment
Number of Bands: In this treatment, no protease is added, therefore the proteins would not be degraded. However, the high salt concentration outside the cell would disrupt the association be. Therefore, we only expect to see proteins B and C.
Position of Bands: No protease was added, so protein B and C were not degraded. These bands should be in the same position as in Lanes 1 and 2.

Lane 5: intact cells + protease treatment
Number of Bands: Protease treatment of intact cells would cause degradation of all protein on the extracellular side of the lipid bilayer. All of protein A is outside the cell, so it would be fully degraded. Proteins B and C are at least partially protected by the lipid bilayer, so we would still expect to see these proteins. Therefore, we can predict there will be two bands in this lane, for protein B and C.
Position of Bands: Protein C is not exposed to the extracellular side of the lipid bilayer, where the protease was present, therefore Protein C should be in the same position as in Lanes 1, 2, 4, and 5. However, Protein B will be partially degraded, as it was exposed to the extracellular side of the lipid bilayer. The band for protein B should therefore be further down the gel than it was in Lane 1, 2, 4, and 5. It is hard to predict how much further down the gel Protein B will appear. While this example shows it above Protein C, it could just as easily be smaller than protein C.

Lane 6: hole in the cell (broken cell) + protease treatment
Number of Bands: As in Lane 5, Protein A would be fully degraded by the protease, and no band will be seen. Proteins B and C are both embedded in the membrane, so while both proteins will be partly degraded, these embedded regions of the protein will be protected. We therefore expect to see two bands, for protein B and C.
Position of Bands: Only the portions of the proteins B and C which are embedded in the membrane will be protected from the protease. The draw suggests that the embedded amount of Protein B is larger than the embedded amount of Protein C. Therefore, we expect that the band for Protein B will be higher up the gel than the band for Protein C. Both bands are expected to be lower than the bands in any other lane, as a greater amount of Proteins B and C are degraded here, than in any other lane.

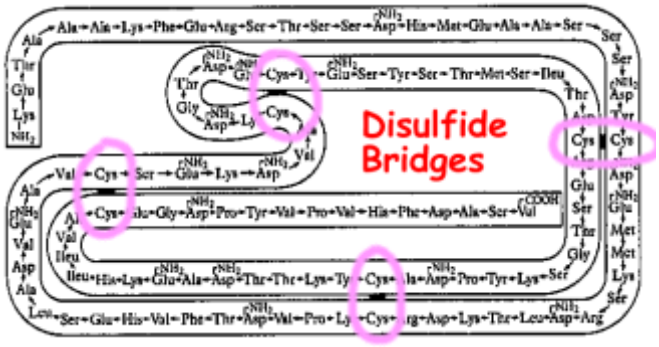
Relating Protein Secondary Structure to Protein Function

Problem 2.1.8

(tags: #protein structure, #animals)

Example Answer: Orange Text
Instructor Comments: Blue Text

The experiment described here is extremely important in the history of cell biology as it provided the first decisive evidence that the folding of proteins was dependent only on the primary structure of the protein and that it was a result of molecular self-assembly (i.e. spontaneous folding based on chemical properties). A Nobel Prize was awarded for this work in 1972.

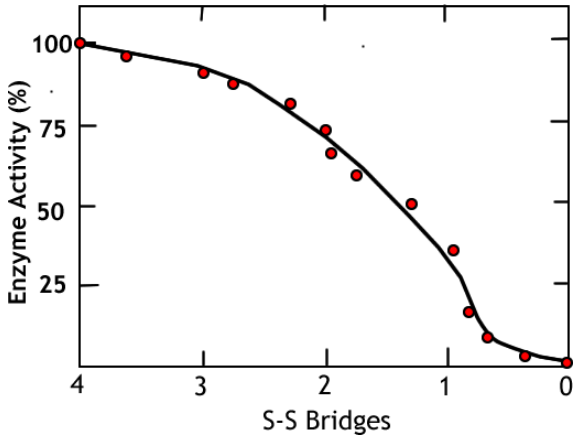


Background: This experiment deals with the structure and activity of ribonuclease, an enzyme that degrades RNA. It is produced in the pancreas. It is one of the first proteins for which the amino acid sequence was known. The protein's enzyme activity occurs only when the molecule is properly folded. As the molecule is denatured, the enzymatic activity is lost.

The molecule has 4 disulfide bonds (C-**S-S**-H) that hold the chain together (see picture on the right). These form between cysteine residues. If the molecule is to be completely denatured, these bonds must be broken. Disulfide bridges are broken by placing the protein in a solution of 8 M urea and beta mercaptoethanol (HS-CH_2-CH_2-OH). The urea interferes with formation of hydrogen bonds and the mercaptoethanol (= reducing agent) provides a reducing environment, in which the disulfide bond will break, leaving 2 –SH groups (one on each cysteine).

The plot on the right (Figure 1) shows how the enzymatic activity decreases as the mean number of disulfide bridges per ribonuclease molecule decreases during denaturation. Each disulfide bridge produces two SH groups:

-C-S-S-C- → C-SH HS-C-
(oxidized) (reduced)



f) Describe how enzyme activity changes as the mean number of disulfide bridges decreases. Why do you think the enzyme activity is affected this way?

Describe the Results: In the first part of this question, you are being asked to demonstrate your correct understanding of the data by describing, in your own words, the results shown in Figure 1. You are simply providing a written representation of the data.

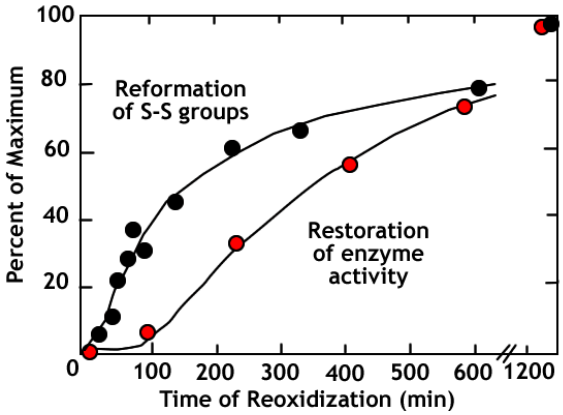
Provide a Cell Biology Interpretation: The second part of this question is asking you to make some connections between the results in the figure and the bigger cell biology impact. What does this data tell us about enzyme activity? What can we infer from the data?

Example Answer:

As the number of disulfide bridges decreases (are broken apart), the % enzyme activity decreases.

This indicates that protein folding is important for enzyme activity.

As the S-S bridges are removed, the molecule unfolds (denatures) and enzyme activity is lost. If the reducing agent and the urea are removed the protein will slowly oxidize in the presence of air. The S-S bonds will reform and enzyme activity is restored, as shown in the Figure 2 on the right.



g) Compare the changes in enzyme activity and reformation of S-S groups change as reoxidization progresses. What does this tell you?

Describe the Results: In the first part of this question, you are being asked to demonstrate your correct understanding of the data by describing, in your own words, the results shown in Figure 2. A good answer will describe both the change in S-S groups and enzyme activity, and how they differ. Be sure to give a thorough description.
Provide a Cell Biology Interpretation: The second part of this question is asking you to make some connections between the results in the figure and the bigger cell biology impact. What does this data tell us about enzyme activity? What can we infer from the data?

Example Answer:

As the S-S bonds reform, the enzyme activity begins to recover, fully recovering by 1200 minutes. We can also see that restoration of enzyme activity lags behind S-S bond reformation.
This tells us that as the S-S groups reform, the enzyme begins to function again.

h) What are some of the assumptions we are making in this experiment?
There are a few assumptions being made.
Example Answer:
We are assuming that the whole protein is refolding properly, but maybe only the active site is still working

i) Why do you think there is a lag between the formation of S-S bonds and the restoration of enzyme activity?
Example Answer:
I think the lag occurs because it takes time for the protein to refold before the enzyme can work properly

j) Based on the data presented here, what did this landmark research contribute to our understanding of protein folding?
Example Answer:
Based on this data, it suggests that the primary amino acid sequence holds all the information needed to fold properly and spontaneously. It also suggests that proteins can be denatured and refolded.

(Source of data: Anfinsen et al. 1961 PNAS 47, 1309, and White 1960 J. Biol. Chemistry 235, 383)

TUTORIAL: Determining Protein Topology Using Protease Digestion and SDS-PAGE

Problem 2.3.8 (Covered in Tutorial, Answer Online)

(tags: #SDS-PAGE, #protein topology)

Unit 3: Nuclear Structure and Function

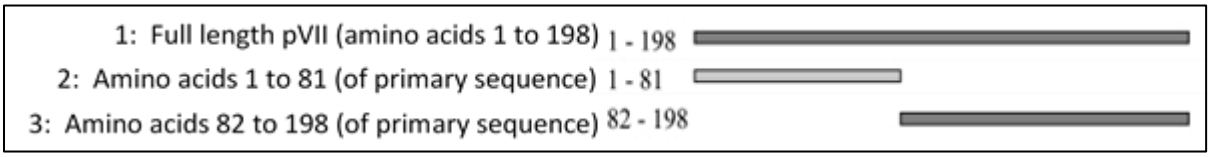
Identifying the Function of Protein Domains by Fluorescence Microscopy of Protein Fragments

Problem 3.1.6

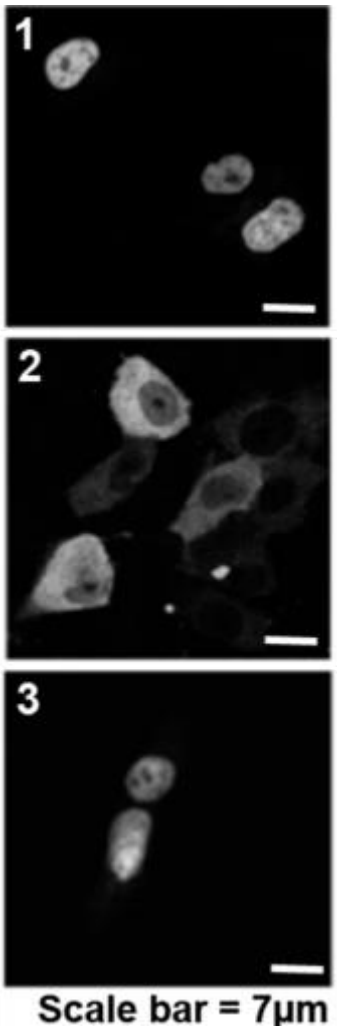
(tags: #fluorescence microscopy, #NLS, #mutant analysis, #animals)

Example Answer: Orange Text
Instructor Comments: Blue Text

Wodrich et al. (2006) looked at the location of nuclear localization signals (NLS) in the Adenovirus protein pVII. Full length and deletion mutants of pVII were fused to GFP (green fluorescent protein) and injected into the cytoplasm of cells. Injected cells were viewed using fluorescence microscopy.



a) Identify the cellular location of the fluorescence in each panel (1, 2 & 3).



Describe the Results: Demonstrate your correct understanding of the data by describing, in your own words, the results shown in the figure.
Example Answer:

- *Panel 1 (construct 1, full length pVII) detected in the nucleus but not the cytoplasm*
- *Panel 2 (construct 2, pVII aa 1-81) detected in the cytoplasm but not the nucleus*
- *Panel 3 (construct 3, pVII aa 82-198) detected in nucleus but not the cytoplasm*

Explanation: This is a bit challenging because no brightfield image is available for comparison, but we can compare the three panels to make things more clear.
Identifying Nuclear Signal: Panel 1 and Panel 3 appear very similar, with labelling of roughly oval-shaped objects approximately 10µm across (approximately the size of a nucleus), often with darker spots inside which may be the nucleolus. The 10um ovals are still visible in Panel 2, but as an absence of fluorescence. Panel 2 also tells us that the cells are much bigger than the oval structures, as it appears that the rest of the cell (each irregular structure is a cell, characteristic of mammalian cell cultures) is being labelled.
Identifying Cytoplasmic Signal: Cytoplasmic signal is generally characterized by a uniform fluorescence throughout the cell, that is excluded from organelles in the endomembrane system and the nucleus. It is not possible to see the exclusion from the endomembrane system in these images, but exclusion from the nucleus is clear.

b) What can you conclude about the location of the NLS in pVII? Explain.

This question is asking us to make some connections between the results above, and the function the different part of the pVII protein. Your job in answering questions like this, is to make these connections as clear, and obvious as possible. In the example below, the 'logical progression' of the answer is clearly laid out.

Example Answer:

- *Conclusion: pVII has an NLS, and it is found somewhere within aa 82-198 of the protein.*
- *Explanation:*
  - *Full-length pVII is detected in the nucleus but not the cytoplasm, indicating that pVII contains a signal for nuclear import somewhere.*
  - *The aa 1-81 protein is found only in the cytoplasm, indicating that there is no signal for nuclear import in this region of pVII.*
  - *The aa 82-198 protein is found only in the nucleus, indicating that this region of pVII contains the signal for nuclear import.*

TUTORIAL: Using Nuclease Digestion to Study How DNA is Packaged

Problem 3.2.2 (Covered in Tutorial, Answer Online)

(tags: #gel electrophoresis, #DNA packing, #nuclease digestion, #experimental controls)

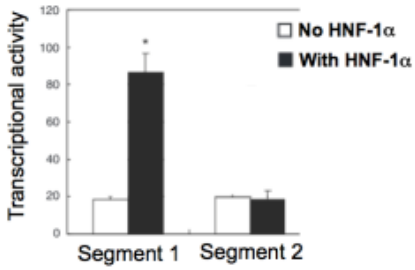
Analyzing Changes in Gene Expression Using Parts of a Transcription Factor

Question 3.3.7

(tags: #gene expression, #mutant analysis, #experimental controls, #prediction, #animals)

Example Answer: Orange Text
Instructor Comments: Blue Text

The *clcn5* gene encodes for a membrane protein that forms a chloride-proton channel in the kidney epithelium. Tanaka *et.al*. (2010) studied how the *clcn5* gene is regulated by analyzing the transcription activity of different segments of DNA upstream of the *clcn5* gene in the presence or absence of a transcription factor HNF-1a. Below is a schematic of the gene and DNA regions they analyzed:



A. What is the control in this experiment? Explain why it is used.
Example Answer:

The control is when no HNF-1a is added. This establishes the basal level of transcription for each segment without the transcription factor HNF-1a. In order to tell what the effect of the transcription factor is on each of the segments, you need to know what the basal level of transcription of each segment is without the transcription factor.

General Suggestion: There are two obvious 'groups' in the bar graph, one for segment 1, one for segment 2. In the design of their figure, the researchers are making it easy to compare the two bars for each segment. Since you know that controls are used to compare experimental groups, you might suspect one of the two bars in each case is a control. In this case you'd be right, but you need to be careful as this is not always the case. Often experimental groups are also compared with each other (i.e. comparing effects of increasing doses of a particular drug).

B. Describe how the transcriptional activity of the Segment 1 and Segment 2 change when HNF-1a is present. What does this tell you about how HNF-1a controls expression of the *ccln5* gene?

Describe the Results: In the first part of this question you are being asked to demonstrate your correct understanding of the data by describing, in your own words, the results shown in the figure.

Example Answer:
Segment 1: *In the presence of HNF-1a there is a 4-fold increase in transcriptional activity compared to the control (No HNF-1a).*
Segment 2: *In the presence of HNF-1a there is no change in transcriptional activity compared to the control.*

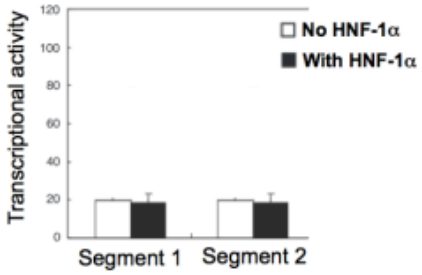
Provide a Cell Biology Interpretation: The second part of this question is asking you to make some connections between the results in the figure and the bigger cell biology impact. What does this data tell us about how cells work?

Example Answer:
***Segment 1**: This tells us that HNF-1a activates transcription of this gene.*
***Segment 2**: This tells us that the region missing in Segment 2 is required for the HNF-1a activation seen in Segment 1.*

C. Predict what would happen to transcription if the first 3 base pairs at the 5' end of Segment 1 were mutated from TCT to AAA. Justify your answer.
Example Answer:
Prediction: Transcriptional activity may stay low even when HNF-1a is present, as this mutation could prevent binding of HNF-1a.
Justification: The data in the figure above shows that the 5'-most end of Segment 1 is necessary for HNF-1a dependent expression of ccln5, as seen when it was deleted in Segment 2.

D. You are working in a lab and you decide to try and repeat the experiment from Question A and B. However, your data do not agree with the published results (see your data below). Your supervisor points out that you were accidentally using cells that lack enzymes that modify core histone proteins by adding acetyl groups. Explain why this omission could explain your result.

Example Answer:
Acetylation of histones can cause loosening of chromatin, making it more accessible to transcription factors like HNF-1a. Therefore it's possible that in these cells the HNF-1a cannot bind to the DNA to promote transcription, as is seen in these new results.



NOTE: This answer is good because it explains why acetylation is important for transcriptional activity, by highlighting the connection between acetylation and chromatin loosening. It also directly answers the question asked by explaining the relationship to the new results.

Unit 4: Endomembranes

Using Protein Domain Diagrams to Predict Topology and Targeting

Problem 4.1.1

(tags: #NLS, #hydrophobicity, #protein targeting, #protein topology, #prediction, #transfer sequence)

Example Answer: Orange Text
Instructor Comments: Blue Text

A. Four proteins are represented schematically below. The numbered boxes represent sequences of hydrophobic amino acids. Predict the cellular location of each protein. (8 marks)

Protein 1: Plasma Membrane

Explanation:

STEP 1: The hydrophobic amino acids act as an ER signal sequence, which directs the protein to the ER during protein synthesis.
STEP 2: The hydrophobic amino acids will become a transmembrane domain, and the C-terminal amino acids will be translocated into the ER lumen.
STEP 3: This protein lacks an ER-retention signal like KDEL, so it will be secreted through the endomembrane system, eventually ending up at the plasma membrane.
NOTE: Because the protein has a transmembrane domain, the nuclear localisation signal (NLS) cannot direct the protein the nucleus.

Protein 2: Secreted out of cell

Explanation:

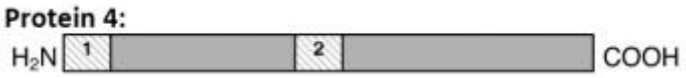
STEP 1: The hydrophobic amino acids act as an ER signal sequence, which directs the protein to the ER during protein synthesis.
STEP 2: The protein is translocated into the ER and the hydrophobic amino acids are cleaved, leaving the rest of the protein in the ER lumen with no transmembrane domain.
STEP 3: This protein lacks an ER-retention signal like KDEL, so it will be secreted through the endomembrane system, eventually secreted from the cell.

Protein 3: ER

Explanation:

STEP 1: The hydrophobic amino acids act as an ER signal sequence, which directs the protein to the ER during protein synthesis.
STEP 2: The protein is translocated into the ER and the hydrophobic amino acids are cleaved, leaving the rest of the protein in the ER lumen with no transmembrane domain.
STEP 3: This protein contains an ER-retention signal like KDEL, so it will stay in the ER.

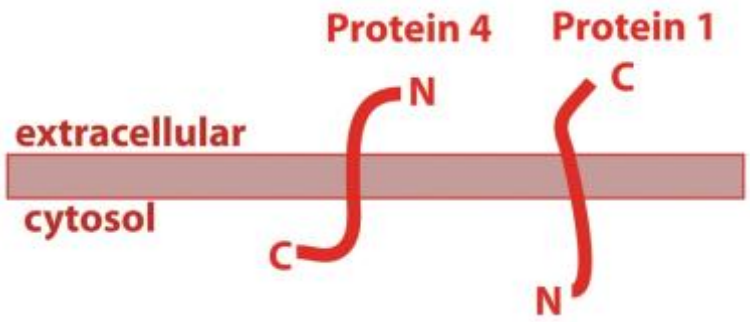
Protein 4: Plasma Membrane



Explanation:

STEP 1: The hydrophobic amino acids at the N-terminus act as an ER signal sequence, which directs the protein to the ER during protein synthesis.
STEP 2: The protein is translocated into the ER and the hydrophobic amino acids at the N-terminus are cleaved. The 'new' N-terminus is not inside the ER.
STEP 3: The second group of hydrophobic amino acids is inserted into the membrane, becoming a transmembrane domain, and the rest of the protein's C-terminus is translocated back into the cytosol.
STEP 4: This protein lacks an ER-retention signal like KDEL, so it will be secreted through the endomembrane system, eventually ending up at the plasma membrane.

B. For proteins 1 and 4 in part A, draw a fully labeled diagram indicating the orientation of the protein in the membrane. (6 marks)



The key differences between these proteins is the location of the hydrophobic amino acids in the protein.

Protein 1: The N-terminus is synthesized in the cytosol, so it will stay in the cytosol. The internal hydrophobic region causes the C-terminus to be translocated into the ER, and eventually the C-terminus ends up on the extracellular face of the cell.
Protein 4: The hydrophobic amino acids at the N-terminus means that this end of the protein is immediately translocated into the ER. After the hydrophobic amino acids are cleaved, the N-terminus is in the ER lumen, eventually ending up on the extracellular face of the cell. The hydrophobic domain in the middle of the protein then causes the C-terminus to be translocated back to the cytosol, where it remains.

To get full marks for this kind of question on an exam, you must fully label your drawing, including the N- and C-termini of each protein. Additionally, since we asked about the destination, you should not be showing these proteins in the ER, but ay their destination site (which is the plasma membrane).

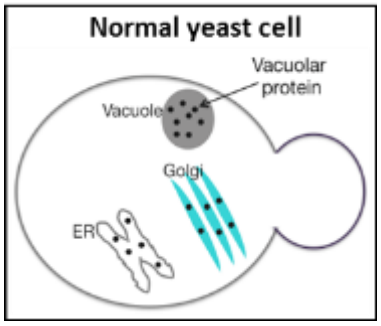
Using Mutant Analysis to Study How Proteins Move Through the Endomembrane System

Problem 4.4.3

(tags: #mutant analysis, #lysosome targeting, #yeast, #protein targeting)

Example Answer: Orange Text
Instructor Comments: Blue Text

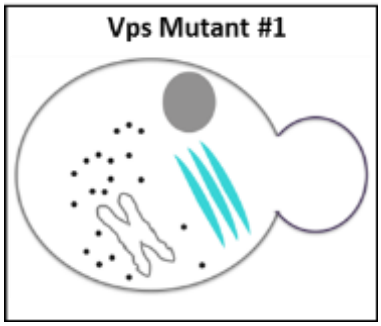
Yeast vacuoles are acidic organelles that are functionally similar to mammalian lysosomes. To identify the machinery that regulates vacuolar protein sorting and transport, scientists have used yeast cells with mutations in vacuolar protein sorting (therefore called *vps* mutants). Below are diagrams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



Mutant Analysis: Mutant analysis is a common method used to study the function of genes and/or proteins. The properties of mutant cells give us clues about what aspect of normal cell biology has been 'broken'. Mutant analysis therefore requires a comparison of the function of the protein in a 'normal cell' and a 'mutant cell'.

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.
(NOTE: there are many possible answers for part B, and any reasonable answer will be accepted. Some good examples are included below)

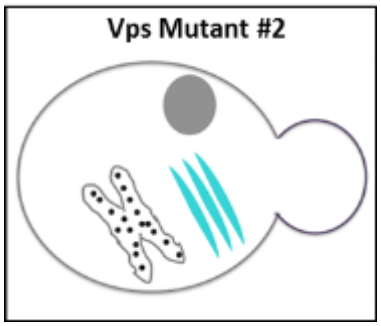
Vps Mutant #1



A. Entry of ER proteins into ER via translocation channel
Explanation: In the normal yeast cells, the protein is found throughout the endomembrane system. To enter the endomembrane system, a protein must first be translocated into the ER. In this mutant, the protein is found in the cytosol, meaning it is this entry into the ER that is disrupted.
B. A few good answers include:

- Signal recognition particle (SRP)
  The SRP binds the ER signal sequence on the ribosome, slowing protein synthesis.
- Signal recognition particle receptor (SRP receptor)
  The SRP receptor is found on the ER membrane. It binds the SRP-ribosome complex.
- Translocation channel
  The ribosome then associates with a translocation channel, through which the protein is transferred across the ER membrane.

Vps Mutant #2



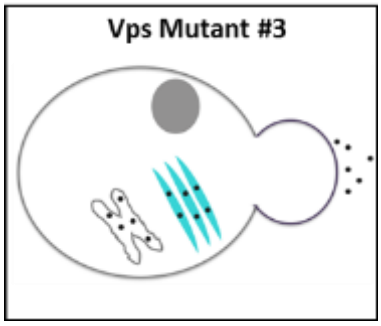
A. Vesicle packaging and/or budding at ER

Explanation: In this mutant, the protein is making it into the ER, but cannot transit through the rest of the endomembrane system. This suggests that the protein is not being packaged into vesicles for transport to the Golgi.

B. A few good answers include:

- Cargo selection/concentration receptors
  The proteins are first concentrated in preparation for vesicle budding by binding to a receptor in the ER.
- COPII
  This coat protein is required for vesicle transport from the ER to the Golgi.
- COP-associated adaptins
  Adaptins are adapters/bridges between the cargo receptors and the coat proteins. They ensure that the cargo and receptors are incorporated into the coated vesicles.

Vps Mutant #3



A. Sorting at trans-Golgi network (TGN) of M6P-tagged proteins to the lysosome

Explanation: In this mutant, the protein makes it to the Golgi, but is secreted from the cell instead of being sent to the lysosome. This suggests that there are problems with mannose-6-phosphate (M6P) targeting of proteins to the lysosome from the TGN.

B. A few good answers include:

- M6P tagging enzymes
  These enzymes are involved in adding the M6P to proteins that need to go to the lysosome.
- M6P receptor
  This receptor binds the M6P on proteins headed to the lysosome, allowing them to be sorted at the TGN and transported properly.

TUTORIAL: Using Radiolabelling, Differential Centrifugation and Mutant Analysis to Study Golgi Processing

Problem 4.3.5 (Covered in Tutorial, Answer Online)

(tags: #Golgi processing, #differential centrifugation, #mutant analysis, #radiolabelling, #experimental controls, #prediction, #animals)

Unit 5: Mitochondria and Chloroplasts

TUTORIAL: Essay Outline (Unit 5: Mitochondria and Chloroplasts)

Problem 5.2.1 (Covered in Tutorial, Answer Online)

(tags: #essay outline)

Relating Protein Targeting Signals to Protein Function

Problem 5.1.1

(tags: #protein targeting, #KDEL, #NLS, #M6P, #signal sequence, #transfer sequence, #chloroplast targeting, #mitochondrial targeting, #CDK, #microtubules)

Example Answer: Orange Text
Instructor Comments: Blue Text

For each protein identified in the table below, choose from the first column the signal(s) the protein would require and write the appropriate NUMBER(S) for the signal(s) in the answer column. Note that each signal may be used more than once, not every signal mentioned may necessarily be used, and not every signal needed may be listed in first column. Marks will be deducted for wrong answers.

These questions require us to first determine where a protein would need to be located in order to carry out its function, and then to determine which signals are required to target a protein to that location.

- Small nuclear ribonucleoprotein (snRNP):
  1: Nuclear Localization Signal
  Location: snRNPs are involved in RNA splicing of introns before the mature RNA exits the nucleus.
  Targeting: Therefore, snRNPs must be targeted to the nucleus, requiring a nuclear localization signal.
- Chloroplast aminoacyl tRNA synthetase (gene in nuclear DNA):
  7: Chloroplast N-terminal targeting sequence
  Location: The question tells us the protein functions in the chloroplast, but is synthesized in the cytosol (like all genes encoded in the nuclear DNA).
  Targeting: In order to reach the chloroplast, a Chloroplast N-terminal targeting sequence is required.
- Mitochondrial ATP synthase (gene in nuclear DNA):
  8: Mitochondrial N-terminal targeting sequence
  Location: The question tells us the protein functions in the mitochondria, but is synthesized in the cytosol (like all genes encoded in the nuclear DNA).
  Targeting: In order to reach the chloroplast, a Mitochondrial N-terminal targeting sequence is required.

- M-CDK:
  1: Nuclear Localization Signal
  Location: M-CDK is a cell cycle protein kinase that triggers the end of mitosis. M-CDKs function in the nucleus.
  Targeting: Therefore M-CDK must contain a nuclear localization signal.
- RNA polymerase III protein:
  1: Nuclear Localization Signal
  Location: RNA Polymerases transcribe RNA from DNA in the nucleus.
  Targeting: Therefore RNA Polymerase III requires a nuclear localization signal (NLS), so it will be targeted to the nucleus.
- Na+/K+ ATPase (integral plasma membrane protein)
  6: Hydrophobic transfer sequence
  OR
  6: Hydrophobic transfer sequence **AND** 2: N-terminal hydrophobic signal sequence
  Location: The question tells us that Na+/K+ ATPase is a plasma membrane protein with at least one transmembrane domain.
  Targeting: A hydrophobic transfer sequence somewhere in the middle of the protein is required for a protein to have a transmembrane domain (requiring signal 6). Signal 2 may also be present, but cannot be the only signal. This is because all N-terminal signal sequences are cleaved as the protein is translocated into the ER. The presence of an N-terminal signal sequence will determine the orientation of the protein in the plasma membrane, but it is not required for it to be targeted there. No additional signals are required to reach the plasma membrane. Once a protein enters the endomembrane system, it transits through the endomembrane system to the plasma membrane, unless an additional signal is present, directing it to another compartment.
- Lipase (lysosomal enzyme)
  2: N-terminal hydrophobic signal sequence **AND/OR** 6: Hydrophobic transfer sequence
  AND
  4: Mannose 6-phosphate on oligosaccharide
  Location: The question tells us that lipase is targeted to the lysosome.
  Targeting: A protein targeted to the lysosome must first enter the endomembrane system. This requires one or more hydrophobic sequences. This translocation can be accomplished with only signal 2, only signal 6, or both signal 2 and 6. The specific signals present would affect the orientation of the protein, but not its targeting to the lysosome.
  This protein would also require a lysosomal targeting signal (signal 4), or the protein would end up at the plasma membrane.
- Alpha tubulin:
  3: No signal
  Location: Alpha tubulin is one of the protein monomers that makes up the cytoskeletal elements called microtubules. The cytoskeleton functions in the cytosol, therefore alpha tubulin must be present here.
  Targeting: All proteins encoded in the nuclear DNA are synthesized and reside in the cytosol, unless they contain some kind of signal sequence. Alpha tubulin, therefore, does not have a signal sequence.

- Zymogen (secreted protein):
  2: N-terminal hydrophobic signal sequence
  Location: The question tells us this is a secreted protein, which must therefore exit the cell.
  Targeting: Proteins the exit the cell via secretion through the endomembrane system. We know that the protein is not present in the plasma membrane, therefore zymogen does not have a transmembrane domain. An N-terminal hydrophobic signal sequence would cause the protein to be translocated into the ER from the cytosol. This signal sequence is then cleaved, leaving the protein in the ER lumen. No additional signals are required, as the zymogen will transit through the endomembrane system, eventually being release from the cell via exocytosis.
- Pho86p (ER resident):
  2: N-terminal hydrophobic signal sequence **AND/OR** 6: Hydrophobic transfer sequence
  AND
  5: KDEL sequence
  Location: This question tells us that Pho86p is found in the ER.
  Targeting: Pho86p must first enter the ER by translocation. Either signal 2, signal 6, or both, would cause this translocation. The specific signals present would affect the orientation of the protein, but not its targeting to the ER.
  As the default location of endosomal proteins is secretion to the plasma membrane, Pho86p requires an additional ER-retention signal, the KDEL sequence.
- Mannosidase (soluble cis-Golgi resident):
  2: N-terminal hydrophobic signal sequence **AND/OR** 6: Hydrophobic transfer sequence
  Location: This question tells us that mannosidase is found in the Golgi.
  Targeting: Mannosidase must first enter the endomembrane system by translocation into the ER. Either signal 2, signal 6, or both, would cause this translocation. The specific signals present would affect the orientation of the protein, but not its targeting to the Golgi.
  Some kind of additional signal would be required for the protein to stay in the Golgi and not be trafficked to the plasma membrane. However, no suitable signal is included in the list.

Unit 6: Cytoskeleton

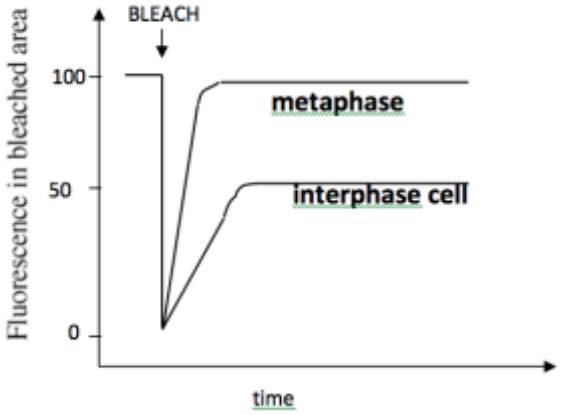
Using Fluorescence Recovery After Photobleaching (FRAP) to Investigate Microtubule Dynamics at Different Stages in the Cell Cycle

Problem 6.2.1

(tags: #microtubules, #FRAP, #cell cycle)

Example Answer: Orange Text
Instructor Comments: Blue Text

One approach to characterize microtubule assembly and disassembly involves injecting fluorescently-labeled tubulin into cells. The fluorescent tubulin is then incorporated into microtubules, thereby allowing visualization of the microtubules inside living cells. When these cells are used in fluorescence-recovery-after photobleaching experiments (FRAP), the following results are found for cells that are in interphase and cells that are in metaphase.



A. When doing FRAP in cells with labeled microtubules, why does the fluorescence intensity in the bleached area recover (i.e. it does not stay at zero)?
A complete answer must connect the principles of FRAP (as displayed in the graph above) to the underlying cell biology of microtubules.

Principles of FRAP: FRAP lets you detect movement of fluorescently-tagged proteins by bleaching the fluorescence and measuring how long it takes for fluorescent proteins to move into the bleached area. If the fluorescence doesn't recover, we can infer that the proteins are not mobile.

Cell Biology of Microtubules: It makes sense that fluorescence intensity is recovered when microtubules are fluorescently-tagged because we know that microtubules are dynamically unstable. The proper functioning of the cytoskeleton requires continual disassembly and assembly. Therefore, there are many opportunities for the tubulin monomers in the bleached area to be exchanged for new, fluorescing tubulin monomers.

Example answer:

> *"Fluorescence intensity recovers because of the dynamic instability of microtubules. While the microtubules with "bleached" tubulin are no longer visible, fluorescently-labeled tubulin from outside the bleached area can move into the bleached area and become incorporated into microtubules, allowing recovery of fluorescence."*

B. What can you conclude about the relative stability of the microtubules in metaphase cells as compared to the microtubules in cells in interphase? Interpret these results in terms of the function of microtubules in metaphase.

This question is asking us to make some connections between the results in the graph above, and the function of microtubules in different stages of the cell cycle. Your job in answering questions like this, is to make these connections as clear, and obvious as possible.

In this case, there are three key points that need to be covered.

1. **Describe the Results**: Demonstrate your correct understanding of the data by describing, in your own words, the results shown in the graph. In this question, you need to compare the fluorescence recovery of microtubules in interphase and metaphase.
2. **Provide a Cell Biology Interpretation**: What do these results suggest about the behavior of microtubules in interphase and metaphase?
3. **Make a Conclusion**: How do these differing behaviors relate to the differing function of microtubules in interphase and metaphase?

Example Answer: "*Fluorescence recovery of microtubules in metaphase is faster than that of microtubules in interphase. This indicates that during metaphase microtubules exhibit a greater degree of dynamic instability (MTs in metaphase cells are turning over faster), and that microtubules in interphase cells are more stable than those in metaphase cells. This reflects the function of metaphase microtubules in capturing chromosomes and maintaining them at the metaphase plate, with increased dynamic instability (and treadmilling).*"

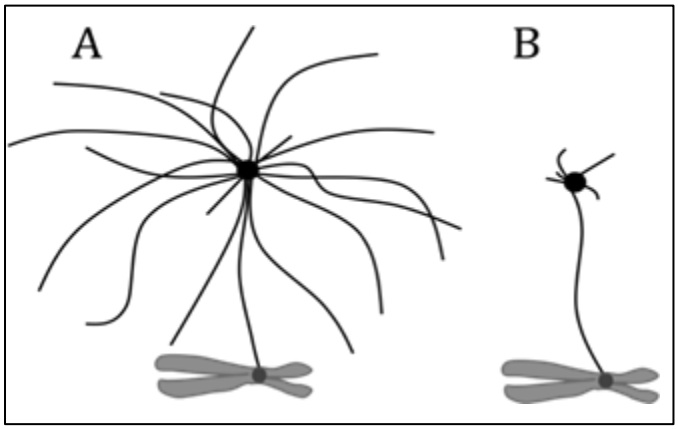
Describe the Results: "*Fluorescence recovery of microtubules in metaphase is faster than that of microtubules in interphase.*"
Provide a Cell Biology Interpretation: "*This indicates that during metaphase microtubules exhibit a greater degree of dynamic instability (MTs in metaphase cells are turning over faster), and that microtubules in interphase cells are more stable than those in metaphase cells.*"
Make a Conclusion: "*This reflects the function of metaphase microtubules in capturing chromosomes and maintaining them at the metaphase plate, with increased dynamic instability (and treadmilling).*"

Investigating the Effect of Tubulin Concentration on Microtubule Dynamics During Mitosis

Problem 6.2.2

(tags: #microtubules, #mitosis, #critical concentration)

Example Answer: Orange Text
Instructor Comments: Blue Text



A classic research study clearly demonstrated the properties of microtubules of the mitotic spindle. Centrosomes were used to initiate microtubule growth *in vitro*, and then chromosomes were added. The chromosomes bound to the free ends of the microtubules, as shown in Fig. A. The mixtures were then diluted to a level below the critical concentration of tubulin and examined again (Fig. B).

A. Describe what happens to the microtubules in A and B.
Describe the Results: Demonstrate your correct understanding of the data by describing, in your own words, the results shown in the figure. In this case, you must compare the results depicted in Fig A and Fig B.

Example Answers:
"Most microtubules shrank at levels below the critical concentrations of tubulin. The microtubule attached to the kinetochore, however, did not shrink"

B. How can we account for the difference between what happens to the kinetochore microtubule and what happens to the other microtubules?
This question is asking us to make a connection between the results in the figure above, and the material from BIOL200 that could explain it. Your job in answering questions like this, is to make these connections as clear, and obvious as possible. In the example below, the 'logical progression' of the answer is clearly laid out.

Example Answer:

- *Microtubule dynamics are altered in response to changes in tubulin concentration.*
- *When the mixture is diluted, the tubulin concentration decreases.*
- *When the tubulin concentration decreases, microtubules are much more likely to shrink, rather than grow.*
- *This agrees with the results for all microtubules in this experiment except the microtubule connected to the kinetochore.*
- *The kinetochore may prevent microtubule disassembly by interacting with the microtubule plus-end and stabilizing it in some way (i.e. acting as a 'cap'*

TUTORIAL: Investigating the Effect of Tubulin Concentration on Microtubule Dynamics

Problem 6.2.8 (Covered in Tutorial, Answer Online)
(tags: #microtubules, #TEM, #critical concentration, #dynamic instability)

Unit 7: Cell Cycle and Mitosis

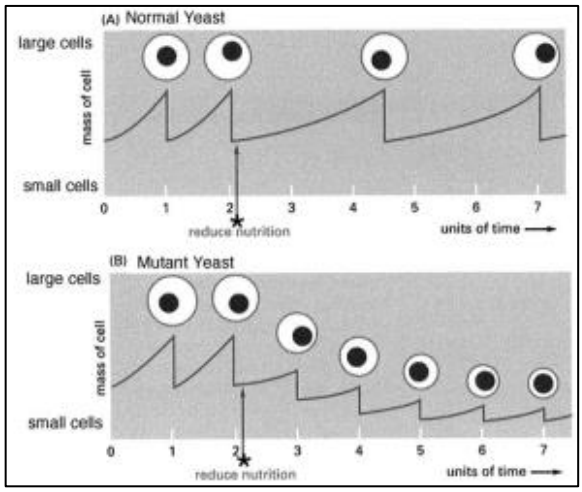
Using Mutant Analysis to Investigate the Effect of Protein Regulation on the Cell Cycle

Problem 7.1.1

(tags: #cell cycle, #drug treatments, #protein regulation, #mutant analysis, #yeast)

Example Answer: Orange Text
Instructor Comments: Blue Text

The diagrams on the right show the relationship between growth rate, cell size, and cell cycle control in yeast. The "mass of cell" refers to the size of an individual cell followed over repeated rounds of the cell cycle, as shown in the drawing of the yeast cell. In the top panel, normal yeast are subject to reduced nutrition (*). In the bottom panel, a mutant is subject to reduced nutrition (*).



A. Describe how decreasing the nutrients available changes the cell cycle and cell size in the normal yeast.

Describe the Results: Demonstrate your correct understanding of the data by describing, in your own words, the results shown in figure (A).

Example Answer:
"Decreasing nutrients cause the cell cycle to become longer. Cell size doesn't change."

B. Describe how decreasing the nutrients available changes the cell cycle and cell size in the mutant yeast.

Describe the Results: Demonstrate your correct understanding of the data by describing, in your own words, the results shown in figure (B).

Example Answer:
"Decreasing nutrients in the mutant causes no change in cell cycle and the cells become smaller."

C. The mutation was found to be in the gene encoding an **inhibitor** protein that binds to G1/S cyclin-dependent kinase and blocks its activating phosphorylation site.
Explain how loss of function of the inhibitor could produce these results. First briefly outline the mechanism of cell cycle control responsible for entry into S in normal yeast, and then indicate why the mutation would cause the observed effect.

This question is asking us to make a connection between the results in the figure above, and the material from BIOL200 that could explain it. Your job in answering questions like this, is to make these connections as clear, and obvious as possible.

Mutant Analysis: Mutant analysis is a common method used to study the function of genes and/or proteins. The properties of mutant cells give us clues about what aspect of normal cell biology has been 'broken'. Mutant analysis therefore requires a comparison of the function of the protein in a 'normal cell' and a 'mutant cell'.

Mechanism For Entry into S:
G1/S Cdk normally acts by:

1. *Binding to cyclin,*
2. *Becoming phosphorylated by kinases on activating and inhibitory sites,*
3. *Becoming dephosphorylated at inhibitory sites, leading to activation.*

Function of G1/S Cdk Inhibitor in a Normal Cell:

- *Low Nutrients:*
  - *When nutrients are low, the G1/S Cdk Inhibitor prevents activating phosphorylation of G1/S CdK*
  - *This G1/S Cdk inhibition prevents the cells from passing the 'Start" checkpoint.*
  - *This delay will allow the cells to reach a proper size before proceeding to S phase.*
- *Sufficient Nutrient:*
  - *If sufficient nutrients are available, the Inhibitor will not prevent G1/S Cdk activation, and the cell cycle will not be delayed.*
- *Therefore, the G1/S Cdk Inhibitor must be part of nutritional sensing system that allows control of the cell cycle if the nutrient is present.*

Function of G1/S Cdk Inhibitor in a Mutant Cell:

- *In the mutant, nutritional status is not sensed because the G1/S Cdk inhibitor is not functional*
- *This means the G1/S Cdk phosphorylation activation site cannot be blocked.*
- *Therefore, the cell cycle proceeds to the next phase, regardless of the level of nutrients.*
- *However, the cell size decreases after nutrition is lowered, because the cells have fewer resources and cannot grow quickly enough to keep up with the uncontrolled cell cycle.*

This is a good answer, because it lays out a logical explanation for all of the data shown in the figure, and references the relevant material from BIOL200.

TUTORIAL: Using Temperature Sensitive Mutants and Drug Treatments to Investigate the Cell Cycle

Problem 7.1.7 (Covered in Tutorial, Answer Online)

(tags: #drug treatments, #mutant analysis, #temperature sensitive mutants, #cell cycle)

Other Skills

Essay Outline (Unit 7: Cell Cycle)

Problem 7.3.1

(tags: #essay outline, #microtubules, #actin, #mitosis, #cell cycle)

Example Answer: Orange Text
Instructor Comments: Blue Text

Here is a sample argument outline question for you to practice with. For this Problem, 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.

> *The process of mitosis depends on interplay between cytoskeleton and chromosomes while the regulation of mitosis entry and exit depends on cytoplasmic proteins. Write an outline that argues the importance of protein activation/deactivation for mitosis (both the process, and the regulation). Provide an overall thesis statement and three supporting arguments (with evidence) to support your arguments.*

There are many possible 'correct' essay outlines, as there are many ways to make a 'good' argument. However here is some information on what we would look for in a 'good' thesis statement, as well as arguments that could be used to support it. Not all correct answers are listed here. See how many others you can come up with.

Organization - 30%

- Flow/Clarity: Marks are lost if erroneous and/or off-topics information is given
- Length/Format:
  - Outline may not exceed 7-8 sentences total.
  - Marks are lost for run-on sentences.
  - Marks are lost if it not in bullet-point (i.e. outline) style
- Spelling/Grammar:

Thesis Statement - 10%

Arguments - 60% (3 arguments, at 20% each)

Thesis statement: **(1-2 sentences MAX)**

Thesis statements should be either 1 long sentence or 2 shorter ones. It should do more than simply restating the question. The most commonly accepted thesis statements reiterated the statement in some way, combined with briefly identifying their 3 arguments. This should be done in a way that is still concise and not overly wordy.

Example Thesis Statement:

"Control of protein activity through protein activation/deactivation is extremely important for mitosis. Entry into mitosis cannot occur without controlled activation of cdk by cyclin, and the separation of chromosomes during mitosis requires control of activation/deactivation cycles of motor proteins, and the tubulin monomers which make up microtubules."

Specific issues that result in loss of marks:

- An incoherent, nonsensical thesis will not merit many marks, even if potentially on-topic
- A thesis that does not address the question asked cannot get full marks.
- A well-written thesis with incorrect or off-topic wrong arguments will lose marks
- An exceedingly long and wandering thesis, with run-on sentences will also lose marks

Arguments and Evidence: **(2-3 sentences MAX, 1 sentence per bullet)**

General Suggestions:

- Unique Arguments: Each argument must provide a DIFFERENT example than the others.
- Arguments and Evidence: Both an argument and evidence supporting the argument are required.
    - Argument: a more general claim, which supports your thesis statement
    - Evidence: more specific evidence that your argument true
- Clarity: Marks will be deducted for incoherency impacting the strength of the argument.

Examples of possible arguments and evidence below.

Please note that there may be more than mentioned here. In all cases we accept anything that is factually correct AND answers the question, regardless of whether we were expecting it as an answer or not.

Arguments and Evidence Examples: **(2-3 sentences MAX, 1 sentence per bullet)**

Specifics for this Question:

- Content: The arguments and evidence must answer the question asked. In this case, they must:
    - Discuss the activation/deactivation state of the protein in question
    - Link this with mitosis
- Types of Arguments: The question asks you to cover BOTH the process AND the regulation of mitosis. Full marks require 1 process argument and 2 regulation arguments, or vice versa.

Arguments related to the **PROCESS** of mitosis:

Microtubules:

Sample Argument:

- *MT dynamics are important for the process of mitosis and are dependent on the activation state of tubulin.*

Sample Evidence:

- *MT dynamics depend on the activation state of tubulin because active tubulin (GTP) can bind to MT and inactive tubulin (GDP) cannot.*
- *MT dynamics are responsible for lining up and pulling apart chromosomes during mitosis*

Microfilaments:
Sample argument:
- *MFs are important in the process of mitosis and are dependent on the activation state of actin.*

Sample Evidence:
- *MF dynamics depend on the activation state of actin because active actin (ATP) can bind to MFs and inactive actin (GDP) cannot*
- *MFs are involved in cytokinesis which lets the cells divide*

Motor proteins:
Sample argument:
- *Movement of motor proteins along cytoskeletal elements is important for the process of mitosis and is dependent on the activation and deactivation state of motor proteins.*

Sample Evidence:
- *Motor proteins (dynein, kinesin, myosin) have an active (ATP) and inactive (ADP) conformation.*
- *Changes in conformation allow them to "walk" along MTs/MFs (cytoskeleton) during the cytoskeleton/chromosome interplay occurring in mitosis.*

M-CDK cyclin Activation:
Sample argument:
- *The activation state of the M-cdk-cyclin complex regulates mitosis by activating target proteins.*

Sample Evidence:
- *H1, lamins, and SRK/SRC have an active and inactive state.*
- *When phosphorylated by the M-cdk-cyclin complex, the DNA condenses, the nuclear envelope breaks down, and the mitotic spindles form.*

Arguments related to the **REGULATION** of mitosis:

M-Cdk Activation/Inactivation: acceptable arguments include…

- Cyclin binding activates Cdk (Cyclin levels are regulated while Cdk levels remain constant)
- Cyclin level is controlled by ubiquitination (induced by anaphase promoting complex APC and proteolysis which results in deactivation of M-Cdk
- Phosphorylation state determines Cyclin/M-Cdk complex activity:
- Activating kinases and Inhibitory kinases, phosphatase that removes the inhibitory P
- Positive feedback to keep cdc25 active, result is to maintain action of M-Cdk

TUTORIAL: Essay Outline (Unit 2: Membrane Fluidity)

Problem 2.1.3 (Covered in Tutorial, Answer Online)

(tags: #essay outline, #membrane fluidity, #animals)

TUTORIAL: Essay Outline (Unit 5: Mitochondria and Chloroplasts)

Problem 5.2.1 (Covered in Tutorial, Answer Online)

(tags: #essay outline)

Index by Key Word

H

I

K

L

M

N

O

V

Y