Questions

Lecture 1

1. What is transfection? What are 3 techniques for transfecting cells with DNA?

2. Explain immunocytochemistry. Briefly explain how it works. What is the difference between immunocytochemistry and in situ hybridization?

3. Describe epifluorescence microscopy, specifically the light path and the use of a dichroic mirror. What is an advantage of fusing a GFP protein to a cell rather than staining for a protein using immunocytochemistry?

4. In light microscopy, if you hold the angular aperature and refractive index constant, but increase the wavelength of light, is the resolving power greater or less?

5. Given an angular aperture of 70 degrees, and the fact that the refractive index of oil is around 1.4, what is the resolving power of a microscope at 488nm wavelength light?

6. What types of antibodies are more specific, monoclonal or polyclonal? Why?

7. What techniques could you use to label proteins? What are advantages of each method?

Lecture 2

1. Briefly define a pulse-chase experiment. Give an example of a pulse-chase experiment used in studying the secretory pathway.

2. List 5 cellular organelles and give their function.

3. Describe the experiment that determined the order of the secretory pathway. Name the 5 classes of mutants and what defect is present in each class.

4. Tay-Sach's disease is caused by a defect in what cellular process?

5. If the carboxyl terminus of a membrane protein is located in the cytoplasm during vesicular transport, where will it the carboxyl terminus be once the protein is inserted into the cell membrane. Intracellular or Extracellular?

6. Peroxisomes degrade molecules primarily through what process?

7. Describe the experiment that determined whether proteins are found in the ER.

8. What are two functions of the golgi?

9. What are two sites where proteins can become glycosylated?

10. How do lysosomes degrade molecules?

Lecture 3

1. Describe one experiment that was performed to determine how proteins are translocated across the ER membrane, and why it is important.

2. Describe the process of getting a protein into the ER, starting with translation at the ribosome and ending with cleavage of the signal sequence.

3. How was it shown experimentally that the translocon is both necessary and sufficient to allow protein translocation into ER microsomes?

4. Describe the SRP. What is it composed of? What does it bind to? What does it do?

5. Why are proteins somewhat shorter inside microsomes?

Lecture 4

1. Describe type I, II, and III membrane proteins. Be sure to include how they are inserted and where the N and C termini are located.

2. Given a Type IV membrane protein, where would the N-terminus be if the protein contained the following components:

C_____SA+++_____+++STA_____SA+++_____+++STA____SA+++____N

3. Why do Type I and Type III proteins share a common orientation yet differ in the length of the N-terminus tail?

4. What is importin?

5. Describe the formation of a GPI-linked protein.

Lecture 5

1. Describe the location and the role of the protein disulfide isomerase (PDI) in protein folding.

2. What is the mannose G-phosphate receptor? What is its function?

3. What is the function of HSC-70, and where is it located?

4. What is the name of the “carrier” protein that aids in exporting proteins from the nucleus?

5. Describe the relationship of the two forms of glutathione in the cytosol.

Lecture 6

1. Describe 3 different defects that lead to familial hypercholesterolemia.

2. Where are two locations that you might find a receptor involved in receptor-mediated endocytosis?

3. What is the role of ApoB?

4. You find that cells contain many LDL particles, randomly distributed across the surface. What is a possible cause of this failure to uptake LDL?

5. In the LDL uptake pathway, what causes the LDL receptor to eventually dissociate from the LDL particle?

6. How do lysosomes maintain their acidity?

Lecture 7

1. Describe the structure of clathrin. What size and # of “faces” are on a clathrin-coated vesicle?

2. How do vesicles identify a particular portion of membrane, and then fuse with it?

3. What are 3 types of intracellular vesicles and where do they travel to/from?

4. COP1 coat proteins are involved in golgi -> ER transport. Why then do proteins build up in the ER?