

## Exercises week 4 BIO-207

1. Imagine that you have engineered a set of genes, each encoding a protein with a pair of conflicting signal sequences that specify different compartments. If the genes were expressed in a cell, predict which signal would win out for the following combinations. Explain your reasoning.

- A. Signals for import into nucleus and import into ER.
- B. Signals for import into peroxisomes and import into ER.
- C. Signals for import into mitochondria and retention in ER.
- D. Signals for import into nucleus and export from nucleus.

2: Discuss the following statement: “the plasma membrane is only a minor component of most eukaryotic cells.”

3: Is it really true that all human cells contain the same basic set of membrane-enclosed organelles? Do you know of any examples of human cells that do not have a complete set of organelles?

4: What is the fate of a protein with no sorting signal?

5: The lipid bilayer, which is 5 nm thick, occupies about 60% of the volume of a typical cell membrane. (Lipids and proteins contribute equally on a mass basis, but lipids are less dense and therefore account for more of the volume.) For liver cells and pancreatic exocrine cells, the total area of all cell membranes is estimated at about 110,000  $\mu\text{m}^2$  and 13,000  $\mu\text{m}^2$ , respectively. What fraction of the total volumes of these cells is accounted for by lipid bilayers? The volumes of liver cells and pancreatic exocrine cells are about 5000  $\mu\text{m}^3$  and 1000  $\mu\text{m}^3$ , respectively.

### 6 TRUE/FALSE

Decide whether each of these statements is true or false, and then explain why.

A) The nuclear membrane is freely permeable to ions and other small molecules under 5000 Daltons.

B) To avoid the inevitable collisions that would occur if two-way traffic through a single pore were allowed, nuclear pore complexes are specialized so that some mediate import while others mediate export.

C) Some proteins are kept out of the nucleus, until needed, by inactivating their nuclear localization signals by phosphorylation.

D) All cytosolic proteins have nuclear export signals that allow them to be removed from the nucleus when it reassembles after mitosis.

Thinking

E) Nuclear localization signals are not cleaved off after transport into the nucleus, whereas the signal sequences for import into other organelles are often removed after import. Why do you suppose it is critical that nuclear localization signals remain attached to their proteins?

7. Match the definition below with its term from the list above.

inner nuclear membrane

nuclear envelope  
nuclear export receptor  
nuclear export signal  
nuclear import receptor  
nuclear Lamin  
nuclear lamina  
nuclear localization signal  
nuclear pore complex  
nuclear transport receptor  
nucleoporin outer nuclear membrane  
Ran

A) Sorting signal contained in the structure of macromolecules and complexes that are transported from the nucleus to the cytosol through nuclear pore complexes.

B) Large multiprotein structure forming a channel through the nuclear envelope that allows selected molecules to move between nucleus and cytoplasm.

C) Monomeric GTPase present in both cytosol and nucleus that is required for the active transport of macromolecules into and out of the nucleus through nuclear pore complexes.

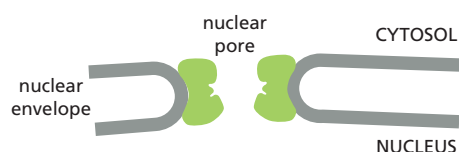
D) Fibrous meshwork of proteins on the inner surface of the inner nuclear membrane.

E) Protein that binds nuclear localization signals and facilitates the transport of proteins with these signals from the cytosol into the nucleus through nuclear pore complexes.

F) Sorting signal found in proteins destined for the nucleus and which enable their selective transport into the nucleus from the cytosol through the nuclear pore complexes.

G) The portion of the nuclear envelope that is continuous with the endoplasmic reticulum and is studded with ribosomes on its cytosolic surface.

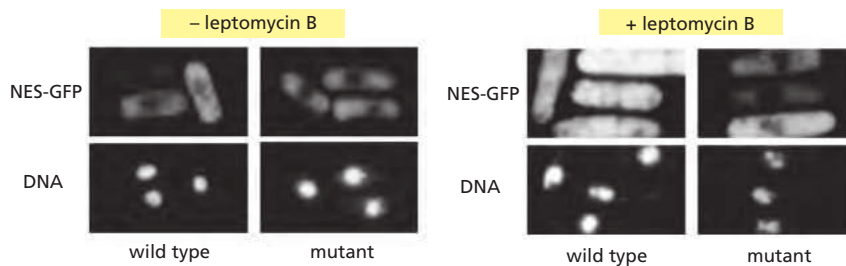
8. As shown in Figure below, the inner and outer nuclear membranes form a continuous sheet, connecting through the nuclear pores. Continuity implies that membrane proteins can move freely between the two nuclear membranes by diffusing through the bilayer at the nuclear pores. Yet the inner and outer nuclear membranes have different protein compositions, as befits their different functions. How do you suppose this apparent paradox is reconciled?



9. How is it that a single nuclear pore complex can efficiently transport proteins that possess different kinds of nuclear localization signal?

10. Nuclear localization signals are not cleaved off after transport into the nucleus, whereas the signal sequences for import into other organelles are often removed after import. Why do you suppose it is critical that nuclear localization signals remain attached to their proteins?

11. The broad-spectrum antibiotic leptomycin B inhibits nuclear export, but how does it work? In the yeast *S. pombe*, resistance to leptomycin B can arise by mutations in the Crm1 gene, which encodes a nuclear export receptor for proteins with leucine-rich nuclear export signals. To look at nuclear export directly, you modify the green fluorescent protein (GFP) by adding a nuclear export signal (NES). In both wild-type and mutant cells that are resistant to leptomycin B, NES-GFP is found exclusively in the cytoplasm in the absence of leptomycin B (Figure below 12–10). In the presence of leptomycin B, however, NES-GFP is present in the nuclei of wild-type cells, but in the cytoplasm of mutant cells (Figure 12–10). Is this result the one you would expect if leptomycin B blocked nuclear export? Why or why not?



**Figure 12–10** Distribution of NES-GFP in *S. pombe* in the presence and absence of leptomycin B (Problem 12–49). *Light areas* in the NES-GFP panels show the position of GFP. *Light areas* in the DNA panels result from a stain that binds to DNA and marks the position of the nuclei in the cells in the NES-GFP panels.

12 A nuclear pore can dilate to accommodate a gold particle 26 nm in diameter. If it could accommodate a spherical protein of the same dimensions, what would the protein's molecular mass (g/mole) be? [Assume that the density of the protein is 1.4 g/cm<sup>3</sup>. The volume of a sphere is  $(4/3)\pi r^3$ .]