Answers week 4

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.
A. The protein would enter the ER. The signal for import into the ER is located at the N-terminus of the protein and functions before the internal signal for nuclear import is synthesized. Once the protein entered the ER, the signal sequence for nuclear import could not function because it would be prevented from interacting with cytosolic nuclear import receptors.
B. Signals for import into peroxisomes and import into ER.
B. The protein would enter the ER. Once again, the N-terminal signal for ER import would function before the internal signal for peroxisome import is synthesized. The peroxisome import signal could not function once the protein was sequestered in the ER.
C. Signals for import into mitochondria and retention in ER.
C. The protein would enter the mitochondria. In order to be retained in the ER, the protein must first be imported into the ER. Without a signal for ER import, the ER retention signal could not function.
D. Signals for import into nucleus and export from nucleus.
D. A protein with signals for both nuclear import and nuclear export would shuttle between the cytosol and the nucleus. Unlike the other pairs of signals, these signals are not necessarily in conflict. A number of cellular proteins, whose function requires shuttling in and out of the nucleus, are designed in just this way.

2 in terms of its functional importance to a cell, the plasma membrane is anything but minor. It is the boundary that separates the cell from the outside world, it controls selective entry and exit of molecules, and it is the principal site at which intercellular communications are received. Only in terms of its surface area and mass is it a minor component, accounting for 2–5% of all the membranes in a eukaryotic cell.

3 While the vast majority of cells in the human body do have a complete set of membrane-enclosed organelles, certain specialized cells do not. A prime example is the red blood cell. At a late stage in its development, the precursor of the red blood cell—the reticulocyte—jettisons all of its inter- nal membrane-enclosed organelles, leaving just the plasma-membrane- enclosed cytosol. he cells that make up the lens of the eye, which lack mitochondria, are similar. But in a way, these are exceptions that prove the rule; these cells are derived from cells that do carry the complete set of membrane-enclosed organelles.

4 In the absence of a sorting signal, a protein will remain in the cytosol.

5 the lipid bilayers in all the membranes in liver and pancreatic exocrine cells would have a volume of 330 μm3 and 39 μm3, respectively, as calculated below for a liver cell:
V = 110,000 μm2 × 5 nm × μm × 0.60 103 nm

V = 330 μm3
Thus, lipid bilayers would account for 6.6% (330/5000) of the volume of a liver cell, and 3.9% (39/1000) of the volume of a pancreatic exocrine cell. For typical cells, then, about 5% of their volume is occupied by lipid bilayers.

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.
True. The nuclear membrane allows free passage of ions and small molecules because it is perforated with numerous nuclear pore complexes— 3000–4000 in a typical mammalian cell—each of which has one or more open aqueous channels through which small water-soluble molecules can passively diffuse.
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.
False. Individual nuclear pores mediate transport in both directions. It is unclear how pores coordinate this two-way traffic so as to avoid head-on collisions.
C) Some proteins are kept out of the nucleus, until needed, by inactivating their nuclear localization signals by phosphorylation.
True. Gene regulatory proteins in particular are subject to this kind of regulation, as a way of preventing gene activation (or repression) until the proper time.
D) All cytosolic proteins have nuclear export signals that allow them to be removed from the nucleus when it reassembles after mitosis.
False. Resident proteins of the cytosol do not have nuclear export signals. They are efficiently excluded from reassembling nuclei by the mechanism of reassembly. The nuclear envelope is initially closely applied to the surface of the chromosomes, excluding all proteins except those bound to
the mitotic chromosomes. Once the envelope is complete, other residents of the nucleus are imported via their nuclear localization signals.
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?
At each mitosis, the contents of the nucleus and the cytosol mix when the nuclear envelope disassembles. When the nucleus reassembles, the nuclear proteins must be selectively re-imported. If the nuclear localization signals were removed upon import, the proteins would be trapped in the cytosol after the next mitosis. By contrast, the contents of other organelles never mix with the cytosol. At mitosis, organelles such as the Golgi apparatus and the ER break up into vesicles, which retain the luminal contents of their larger parents. Because of this, their resident proteins have to be imported only once, and their signal sequences are therefore dispensable.

7.
A) Nuclear export signal

B) Nuclear pore complex (NPC)
C) Ran
D) Nuclear lamina
E) Nuclear import receptor
F) Nuclear localization signal
G) Outer nuclear membrane

8 Two aspects of protein function may contribute to the difference in the protein compositions of the inner and outer nuclear membranes. First, proteins that function in the inner membrane are usually anchored by their interactions with components of the nucleus such as chromosomes and the nuclear lamina, which is a protein meshwork underlying the inner nuclear membrane. Freely diffusing proteins that are anchored once they reach the inner membrane would accumulate there. Second, proteins that form the nuclear pore itself may restrict the free diffusion of other membrane proteins by virtue of their insertion into the lipid bilayer at the boundary between the inner and outer membranes. Any mem- brane protein that cannot pass through the ring of nuclear pore proteins would be restricted to the outer membrane.

9 A single nuclear pore complex can transport proteins with quite difer- ent kinds of nuclear localization signal because transport is mediated by a variety of nuclear import receptors that are encoded by a family of related genes. Each family member—each gene product—is specialized for transport of a group of nuclear proteins that share structurally similar nuclear localization signals. At the same time, all family members share common features that allow them to interact with nuclear pore com- plexes. hus, nuclear import receptors act as adaptors between proteins with diverse nuclear localization signals and the uniform population of nuclear pore complexes.

10 At each mitosis, the contents of the nucleus and the cytosol mix when the nuclear envelope disassembles. When the nucleus reassembles, the nuclear proteins must be selectively re-imported. If the nuclear localiza- tion signals were removed upon import, the proteins would be trapped in the cytosol after the next mitosis. By contrast, the contents of other organelles never mix with the cytosol. At mitosis, organelles such as the Golgi apparatus and the ER break up into vesicles, which retain the luminal contents of their larger parents. Because of this, their resident proteins have to be imported only once, and their signal sequences are therefore dispensable

11 These results are more or less what you would expect if leptomycin B blocked nuclear export. In the absence of leptomycin B, NES-GFP is excluded from the nuclei, as shown by the dark areas that correspond to the positions of the nuclei in the DNA panels (see Figure 12–10). his result indicates that NES-GFP is eiciently exported from nuclei. (Of course, it could also mean that NES-GFP never entered the nuclei in the irst place.) he same result is observed in leptomycin B-resistant cells in the presence of leptomycin B, as expected if it is without efect in the mutant cells. he presence of NES-GFP in the nuclei of wild-type cells treated with leptomycin B conirms that NES-GFP can enter the nucleus and that leptomycin B prevents its export. he presence of NES-GFP in the cytoplasm, as well, indicates either that NES-GFP doesn’t enter the nucleus very well or that leptomycin B doesn’t completely block nuclear export.

12 he volume of a spherical protein 26 nm in diameter is 9200 nm3 [(4/3) × 3.14 × (13 nm)3 = 9198 nm3]. he molecular mass of a protein with this volume is 7.7 × 106 g/mole.
molecular mass = 9200 nm3 × 6 × 1023 molecules × cm3 × 1.4 g molecule mole 1021 nm3 cm3
= 7.7 × 106 g/mole (equivalent to about 70,000 amino acids of average molecular mass, 110 g/mole)
As this calculation indicates, a nuclear pore can dilate to accommodate very large proteins.