ANSWER KEY

Name:_____ SID:_____

Question 1 (18 points) – Organization of the cell

(4 points) Name two organelles in animal cells that are enclosed by two membranes.

nucleus, mitochondria

(6 points) Cellular dimensions: In each case, circle the number that is closest to:

a) The size of a mammalian red blood cell:
0.7μm (7 μm) 70 μm

b) The thickness of a lipid bilayer:
(5 nm) 50nm 500nm

c) The size of the selectivity filter of the K^+ channel:
0.1nm (1nm) 10nm

(8 points) Are the following statements 'True' or 'False'

a) All ribosome subunit assembly occurs in the nucleolus

False (not true for mitochondrial ribosomes)

b) Cells that make mostly secreted proteins have a lot of smooth endoplasmic reticulum

False

c) The mitochondria in bacterial cells have their own DNA

False

d) The nucleolus is separated from the remainder of the nucleus by a membrane

False

ANSWER KEY

Name:_____ SID:_____

Question 2 (18 points) Nucleocytoplasmic transport, methods in cell biology:

A drug that specifically blocks the interaction of exportin with all cargoes is added to tissue culture cells. Shortly after addition of the drug, what would the effects be on:

a) (2 points) The localization of newly assembled ribosomal subunits

Accumulate in nucleus

b) (2 points) Export of mRNAs from the nucleus

No effect

c) (2 points) The export of importin from the nucleus to the cytoplasm

No effect

(6 points) Since there is mixing of nuclear and cytoplasmic proteins during mitosis, how is the nucleocytoplasmic gradient of Ran-GTP re-established when the nuclear envelope reassembles after mitosis?

Ran-GEF associates with chromosomes / chromatin during mitosis (2 points)

Ran-GAP is not associated with chromosomes / chromatin (2 points)

When the nuclear envelope forms around chromosomes after mitosis, ran-GEF is in the nucleus + ran-GAP is in the cytoplasm → ran-GTP gradient re-established (2 points)

(6 points) In what way can we study trafficking in cells with green fluorescence protein (GFP) that we could not do previously with electron microscopy? In what way has it changed our view of cell structure?

GFP is intrinsically fluorescent and can be visualized in live cells (no fixing or addition of substrate) (4 points)

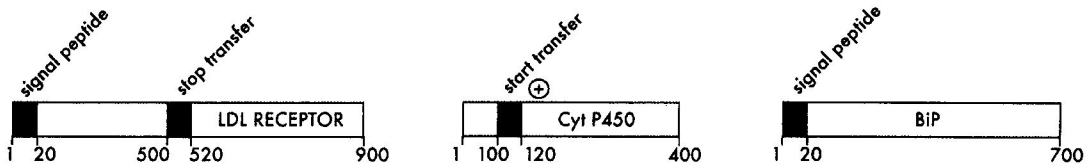
Many cellular structures are more dynamic than previously realized (2 points)

ANSWER KEY

Name:_____ SID:_____

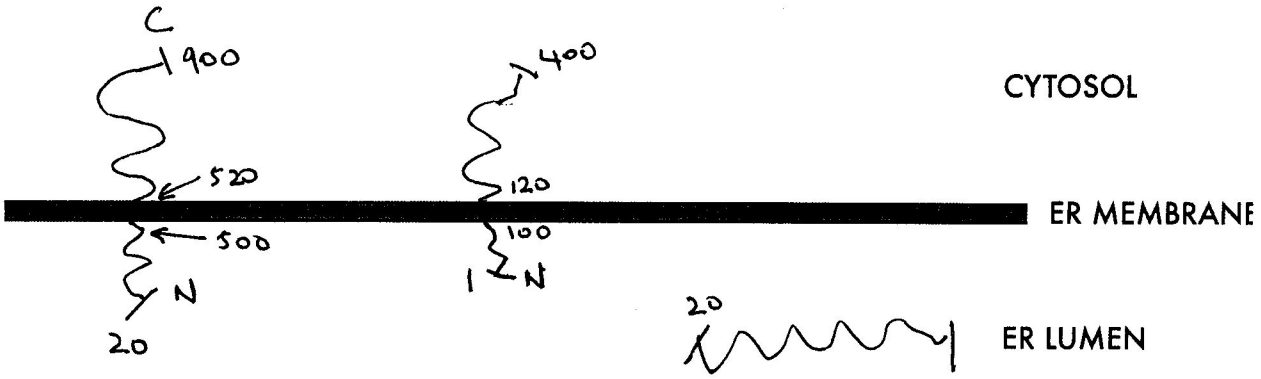
Question 3 (18 points) Membrane proteins

a) (9 points) Use the membrane diagram below to draw how the mature LDL receptor, cytochrome P450 and BiP are arranged within the ER membrane and the ER lumen. Indicate the amino- and carboxy-terminus of each protein.



[Note: numbers indicate amino acid positions]

1.5 points for correct localization (single-pass TM)
1.5 for correct orientation



1 point for correct localization*
1 point for correct orientation
1 point for cleavage of signal peptide

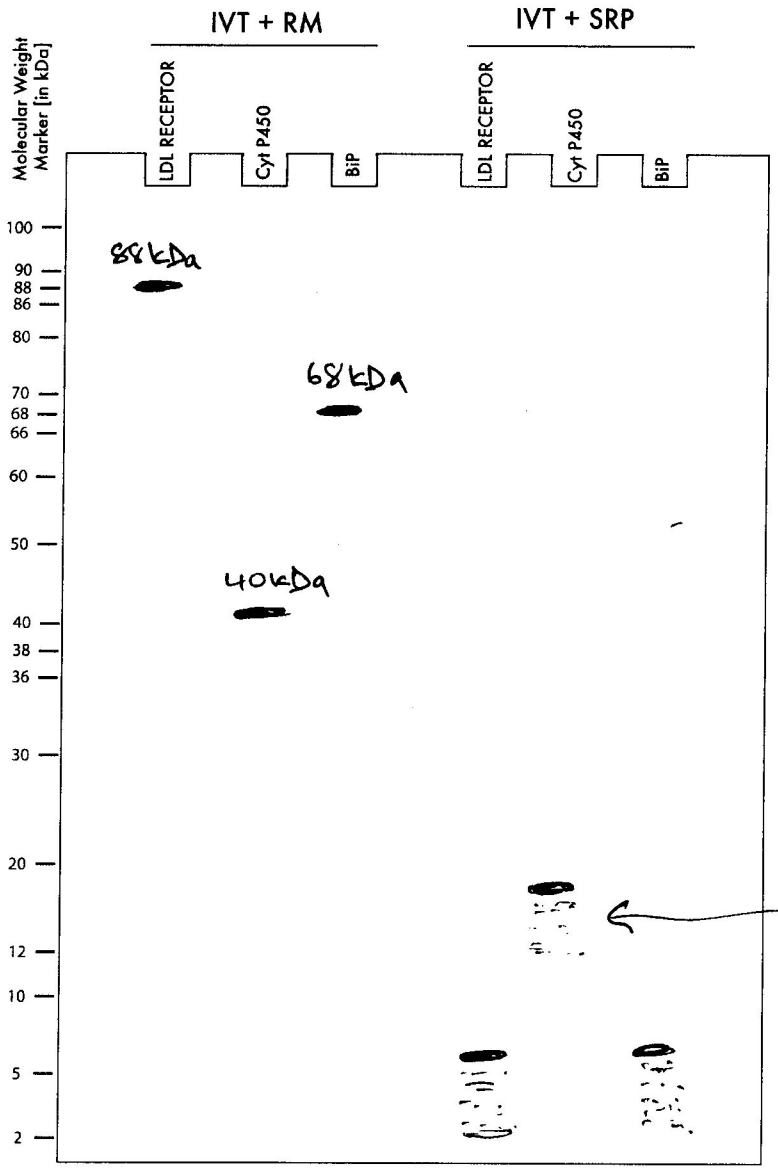
1.5 points for correct localization
1.5 points for cleavage of signal peptide

* i.e single-pass transmembrane protein

ANSWER KEY

Name:_____ SID:_____

b) (9 points) Protein translocation of LDL receptor, cytochrome P450 and BiP is studied in an in vitro translocation assay. The assay takes advantage of (1) in vitro translation reactions (IVT) in the presence of rough microsomes (RM), or (2) in vitro translation reactions (IVT) in the presence of purified SRP (no rough microsomes). Assume that all factors necessary for translation are present in both cases. In each case, the proteins obtained are separated on a gel (SDS-PAGE). Use the gel below to indicate the position *and* label the molecular weight of each of the translation products that you expect in each reaction. For simplicity, assume that each amino acid has a molecular weight of 100 Da.



Any answer between 12 + 16 kDa is acceptable

} any answer between 2 and 6 kDa is acceptable

(No partial credit for this question 1.5 points for each correct answer.

ANSWER KEY

Name:_____ SID:_____

Question 4: (18 points) Vesicle trafficking

Are the following statements 'True' or 'False'?

a)(2 points) Transmembrane proteins that are resident in the ER have a KDEL motif

False

b) (2 points) Dynamin is required for COPII-coated vesicles to bud off the ER

False

c) (2 points) Patients with I-cell disease secrete all of their lysosomal enzymes into the extracellular fluid

True

d) (2 points) Individual clathrin-coated vesicles that form at the plasma membrane can simultaneously contain both LDL-receptor and transferrin receptor molecules

True

e) (2 points) The binding of v-snares and t-snares is facilitated by NSF and ATP hydrolysis

False

f) (4 points) Compare what happens to the LDL-receptor and its ligand with what happens to the transferrin-receptor and its ligand in the low pH of the endosome.

LDL dissociates from the LDL receptor in the endosome.
Transferrin remains bound to the receptor but releases the Fe bound to it.

g) (6 points) Explain the cisternal maturation model of trafficking cargoes through the Golgi apparatus i.e. in this model, what happens to cargoes and what happens to resident Golgi enzymes?

3 key points

(2 pts) Cisternae remain relatively intact but change in character progressively from cis → medial → trans Golgi.

(2 pts) Most cargoes remain within the cisternae as they mature

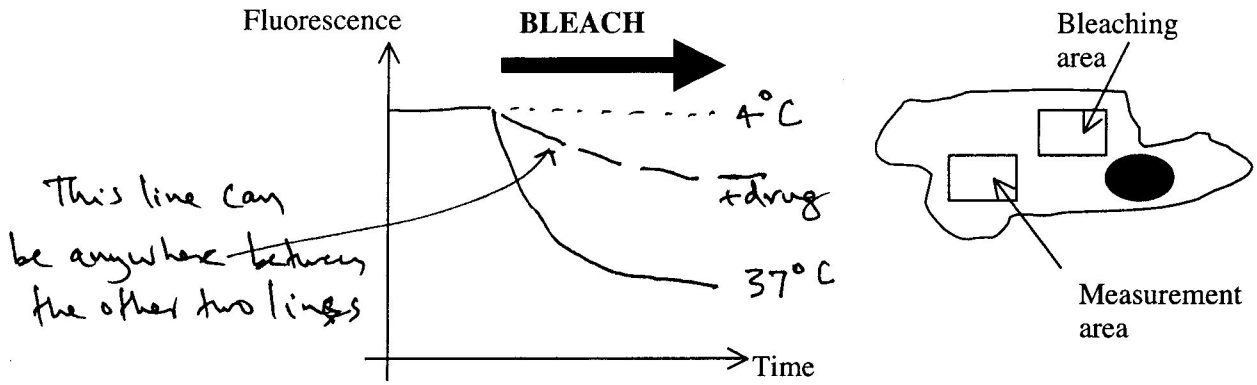
(2 pts) Enzymes that are resident in each Golgi compartment are transported in vesicles to cisternae that follow behind - this changes the characteristics of the cisternae.

ANSWER KEY

Name:_____ SID:_____

18

Question 5: (~~14~~ points) Putting it all together....
You perform a FLIP (fluorescence loss in photobleaching) experiment on a cell where the low-density lipoprotein receptor (LDL receptor) is fluorescently labeled. You bleach and measure from different regions of the cell membrane.



a) (2 points) On the axes provided, draw the result you would expect for a FLIP experiment conducted at 37°C. Label this line '37°C'.
b)(2 points) On the same axes, draw the result you would expect for a FLIP experiment conducted at 4°C. Label this line '4°C'.
c)(6 points) On the same axes, draw the result you would expect if the experiment was done at 37°C in the presence of a drug that inhibits dynamin function. Label this line '+ dynamin' Explain why this line is or is not the same as the lines drawn in either (a) or (b)

The dynamin inhibitor ~~Dynamin~~ will block the movement of receptors from one region to the other via endocytosis and recycling but not by lateral diffusion (4 pts for explanation 2 pts for line)

d) (8 points) What happens to LDL receptor levels in cells if the cells had been treated with a statin? (increase, decrease or unchanged). Explain the mechanism by which statins would have this effect.

(2 pts) Statin → Inhibit HMG-CoA reductase & reduce intracellular cholesterol.

(2 pts) ↓ cholesterol causes dissociation of Insig-SCAP-SREBP complex in ER + SCAP-SREBP goes to the Golgi

(2 pts) Cleavage of SREBP in the Golgi by intramembrane proteolysis results in translocation of part of SREBP to the nucleus

(2 pts) This portion of SREBP activates transcription of the LDL receptor gene → ↑ LDL receptor levels.

MCB 130 Midterm 1

Fall 2008

Instructor: Iswar Hariharan

$\bar{X} = 58.9$

S.D. = 12.3