MCB110
Second Midterm

April 8, 2010

_____.
Your name and student ID

QUESTION	POINTS
1 (10 points)	
2 (10 points)	
3 (15 points)	
4 (20 points)	
5 (15 points)	
6 (20 points)	
7 (20 points)	
8 (20 points)	
9 (20 points)	

TOTAL (150 points)

WARNING: Your exam will be taken apart and each question graded separately. Therefore, if you do not put your name and ID# on every page or if you write an answer for one question on the backside of a page for a different question, you are in danger of irreversibly LOSING POINTS!

Spring 2010 – Second Midterm

Name_____ SID _____

Q1 – Describe clearly but concisely what Fluorescence Recovery After Photobleaching (FRAP) is (6 points). If you use this methodology to visualize two integral membrane proteins in vivo, one resident in the nuclear inner membrane and tightly attached to nuclear lamins, and one in the plasma membrane that does not interact with the cytoskeleton, what are the differences in behavior you will see (4 points)

In FRAP a region in the cell containing fluorescence (lightly via GFP genetically attached to your protein of interest) is bleached with an intense laser to eliminate fluoresce locally. Then the change in fluorescence intensity in that area is measured as a function of time. For a protein diffusing the intensity should recover, as proteins from outside the bleached are move into it. The speed of the recovery carries information on the diffusion rate. For proteins that cannot diffuse, no recovery of fluorescence will be seen. For the two examples, the first will show no recovery of fluorescence, while the second one will.

Q2 –Describe in one short sentence each, what special step will be required to separate from the plasma membrane: 1) an integral membrane protein, 2) a peripheral membrane protein, 3) a lipid-anchored protein (6 points) In the first case, what kind of information will you be able to get about the protein by analysis of its hydrophathy plot? (4 points)

1) **solubilization with mild, non-ionic detergents.**
2) **Alkaline solution to disrupt polar interactions**
3) **Lipases to break a covalent bond linking the soluble domain to the lipid or alkyl chain.**

The hydrophathy plot of the integral membrane protein will tell me if it contains transmembrane alpha helices and how many.

Name_____ SID _____

Q3 – Briefly explain how voltage-gated ion channels are able to sense and respond to changes in electric potential across the membrane (15).

Voltage-gated channel have a structural element that contains positively charged amino acids and whose position/conformation changes when the electric potential changes. These changes allosterically affect the gate region of the channel resulting in its closing or opening.

Q4 – Describe the structural steps in the ATP cycle of the Ca++ ATPase that lead to the pumping of Ca2++ out of the cell. (20).

In the E1, unphosphorylated state, the ATPase is open to the cytosol and has very high affinity for Ca++. So, in spite of the low intracellular Ca++ concentration, it binds Ca++.

Binding of Ca++ triggers the binding of ATP and its hydrolysis, which results in the phosphorylation of the pump and its switch to the E2, phosphorylated conformation.

In the E2 conformation the pump is open to the extracellular space. It has very low affinity for Ca++ ,so the Ca++ ions are released in spite of the higher concentration there.

The pump then losses the phosphate and switches back to the E1 state. The pump, now open to the cytosol in its E1 state, is able to restart the cycle.

Name_____ SID _____

Q5 – Describe and explain two essential differences between ion channels and ion pumps that relate to speed and directionality (15 points)

Movement of ions through channels is extremely fast because once opened the channel lets millions of ions through without requiring any conformational change, acting as a passive hole in the membrane. In contrast, pumps need to bind ATP, hydrolize it, and undergo significant conformational changes in order to let one or two ions move across the bilayer. This takes a long time and the rate of ion movement is very slow.
Ion channels are bidirectional, and ions move down them in the direction determined by the electrochemical gradient. Pumps work in only one direction, which is determined by the set of conformational changes and accompanying changes in affinity for the ion during the ATPase cycle.

Q6 – Describe the molecular players and the distinctint steps involved in the cotranslational translocation of an integral membrane protein with an N-terminal signal sequence and a stop-transfer anchor sequence in the middle of the protein (15 points) What topology will the protein have on the plasma membrane (5).

The signal sequence, as it comes out of the ribosome will be recognized by the SRP that will pause translation until it interacts with its receptor on the ER. The interaction will bring the signal sequence in contact with the translocon, which will then open to let in the signal sequence, and releasing the SRP for another round of capture.
The protein will be translated and translocated into the ER lumen until the STA is translated, reaches the translocon and is translocated into the membrane bilayer to form a transmembrane alpha helix. The rest of the protein will be translated and ultimately release in the cytosol.

The topology of the protein in the PM will be an extra cellular N-terminal domain, followed by a transmembrane helix, followed by a cytosolic C-terminal region. (drawing will also work)

Name_____ SID _____

Q7 –During the process of clathrin-mediated endocytosys,

(a) What is the role of clathrin? (5)

To deform the membrane to allow the budding process

(b) What is the role of adaptor proteins and ligand receptors?(5)

Receptors bind the ligands and adaptors bind the receptors and clathrin, thus ensuing that the formation of a vesicle incorporates selectively the ligand.

(c) What is the role of dynamin? (5)

Pinching of the clathrin-coated vesicle off the plasma membrane.

d) How is clathrin recycled? (5)

Clathrin disassembles after budding off from the plasma membrane and the soluble tryskelions reassemble at the site of coated pits.

Name_____ SID _____

Q8 – In the response of a muscle cell to adrenaline (epinephrine),

(a) How is the signal amplified? (10)

1- While epinephrine is bound to its receptor, this activates numerous G-proteins.
2 - Each activated G-protein activates one adenylyl cyclase while bound to GTP. During that time each enzyme produces many cAMP molecules.
3 – 4 cAMP activate 2 PKAs, and each PKA phosphorylates several down stream targets.
4 – One of the targets of PKA is phosphorylase kinase. Each phosphorylase kinase activates many phosphorylase molecules.
5 – Each phosphorylase, enzymatically, generates many glucose molecules from glucagon.

(further details are OK, but do not add more points)

(b) How is the signal terminated (10)

1 - After epinephrine disappears the receptor becomes inactive
2 – G-proteins hydrolize GTP and cannot be reactivated by the receptor, so adenylyl cyclase becomes inactive
3 – cAMP is degraded and no more produced
4 – PKA losses its cAMP as the levels decrease and becomes deactivated
5 – Inactivation of PKA results in deactivation of inhibitor-1
6 – Inactivation of inhibitor-1 results in the activation of phosphatase-1, which dephosphorylates the proteins in the pathway.

Name_____ SID _____

Q9 – In the response to insulin which roles do these molecules play (20):

a) Insulin receptor

Binds insulin and autophosphorylates, generating phospho-tyrosine sites on itself and different substrates

b) IRS proteins

They are adaptor proteins that contain SH2 domains that bind to phosphotyrosine sites, and additional domains that interact and bring to the membrane some effector proteins, such as PIP3-kinase

c) PIP3

Serves to bind to the membrane and localize/activate down stream effectors, included PIP3-dependent protein kinase

d) Concerning glucose metabolism, how do glucagon and insulin have opposite effects?

Glucagon promotes glycogen breakdown and stops glucose synthesis. Insulin promotes glycogen polymerization and glucose synthesis