MCB110
FINAL

May 17, 2008

_____.
Your name and student ID

QUESTION	POINTS
1 (15 points)	
2 (20 points)	
3 (15 points)	
4 (10 points)	
5 (20 points)	
6 (15 points)	
7 (25 points)	
8 (25 points)	
9 (15 points)	
10 (40 points)	
11 (50 points)	
12 (50 points)	

TOTAL (300 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!

Name_____ SID _____

Q1 – There are two main structural motives for integral membrane proteins, one exemplified by bacterial porins and another by GPCRs. Briefly describe each one (5 points). Which one can be predicted using hydropathy analysis and why? (10 points)

The two motives are beta barrels (porins) and transmembrane helices, either one or a bundle (GPCRs). In the first one, hydrophobic and hydrophilic amino acids alternate along each beta strand. In alpha helical transmembrane segments, a long stretch of 25 or more hydrophobic amino acids is used to traverse the hydrophobic core of the bilayer. These stretches can be detected in hydrophathy plots and thus predicted from the sequence of the protein.

Q2 – The diffusion of integral membrane proteins in the plasma membrane can be demonstrated using cell fusion experiments and fluorescently label antibodies for the extra cellular regions of an integral membrane protein. Can you please describe such an experiment and the results for either a freely diffusing protein versus one that is anchored to the cytoskeleton and thus unable to diffuse? (10 points) In the first case, can you predict what the effect of lowering the temperature in the experiment would do to the quantitative outcome? (10 points).

The classical experiment uses antibodies specific for two integral membrane proteins that are present in human and mouse cells, respectively (GRADERS, THESE KIND OF DETAILS ARE NOT ESSENTIAL BUT NICE). Each one is fluorescently label with different colors and added to a mixture of human and mouse cells, so one type of cell would be label red and the other green (say). When cell fusion is promoted (by addition of a virus), the fusion of a human and mouse cells would be seen initially as a larger entity with two separated colors in the two hemispheres. If the proteins under study are free to diffuse the colors would intermingle and the cell will appear yellow (say). Otherwise the two colors will remain separate. In the first case the diffusion speed depends on the fluidity of the membrane. If the temperature is lowered the fluidity will be reduced and the mixing will take longer

Name_____ SID _____

Q3 – Voltage-gated ion channels typically become inactivated after about 1 msec following opening of the gate. What structural element is responsible for this inactivation, how does it work, and how was the element and its overall mechanism tested by an experiment described in class (15).

Inactivation occurs via an N-terminal globular domain connected to the rest of the channel by a long linker. It is believe to work by a "ball-and-chain" mechanism, where following the opening of the gate the channel gains affinity for this globular domain (ball) which binds to the open channel blocking it. Reactivation then involves the loss of affinity for the domain, which then disengages, allowing for a new round of activation. The function of the segment was tested using a mutant that lucked this region. The channel would open upon voltage change but would be incapable of inactivation and would remain open as observed by electrophoretic current reading through the channel. Upon addition of the segment in trans to the cytosolic compartment, slow, but robust inactivation was then observed following gate opening.

Q4 – Explain the physical bases for potassium selectivity based on the crystal structure of the open potassium channel. In particular, why does the channel not allow Na+ through, even thought sodium ions are smaller? (10)

The structure of the p-segment or selectivity filter is such that main chain carbonyl oxygens of the four chains define a narrow pore and are positioned to simulate the geometry of the oxygen atoms in water molecules fully hydrating a potassium ion in solution. Because sodium is smaller, these carbonyl oxygens are too far apart to optimally compensate for the energy of water hydration in solution.

Name_____ SID _____

Q5 – Describe the structural steps in the ATP cycle of the Na/K ATPase that lead to the pumping of three Na+ ions out of the cell and two K+ ions into of the cell. Pay particular attention to describing the changes in affinity for the two ions that allow for the formation of high concentration gradients (20).

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

Binding of Na 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 Na so the Na ions are release, in spite of their high concentration. The pump has now high affinity for K and, in spite of their low concentration outside the cell, two K ions bind.

Binding of K results in the loss of the phosphate and the switch back to the E1 state. In this conformation the affinity for K is very low, so K ions are release, in spite of their high intra cellular concentration.

The pump, now open to the cytosol in its E1 state, is able to restart the cycle.

Q6 – The epithelial cells of the intestine need to concentrate glucose and then release it into the blood steam. The process requires three distinct transporters: a pump, a facilitative transporter and a co-transporter. Name and describe the activity of these transporters and indicate where they are located in the cell to ensure proper physiology (apical versus basal surfaces) (15).

A glucose-Na co-transporter in the apical membrane facing the lumen of the intestine couples the movement of Na ions down their concentration gradient (into the cell) to the movement of glucose into the cell, creating a concentration of glucose that is higher in the cell than in the intestine. The Na/K ATPase, in the basal surface facing the blood stream, generates the needed Na concentration gradient by pumping Na out of the cell, while pumping K into the cell. A glucose facilitative transporter in the basal membrane allows the passage of glucose out of the cell and into the blood stream by passive diffusion down its concentration gradient.

Name_____ SID _____

Q7 – An a neuronal synapse the action potential is converted into a chemical signal that diffuses through the synaptic cleft and potentially initiates a new action potential in the postsynaptic cell.

(a) How does the arrival of the action potential at the axon terminal result in the fusion of neuro-transmitter containing vesicles to the presynaptic membrane? (5)

The action potential opens voltage-gated Ca channels that let Ca into the cell. The rise in Ca results in the fusion of the vesicles to the plasma membrane

(b) How does a neuro-transmitter like acetocholine activate an action potential in a postsynaptic cell? (10)

By binding to a ligand-gate Na channel. Binding results in opening of the channel and influx of Na, initiating a new action potential.

(c) How is the signaling by a neuro transmitter terminated at the synapse (two possibilities need to be mentioned) (5)

It has to be removes, either by reuptake thought endocytosys into the presynaptic cell, or by enzymatic degradation.

(d) How it is possible for a neuro-transmitter to bind to its ligand-gated channel in the postsynaptic membrane and result in hyperpolyrization rather than depolarization of the postsynaptic membrane? (5)

By binding to a ligand gated channel for Cl.

Name_____ SID _____

Q8 – Predict the phenotypic effect in the biosynthetic pathways of temperature-sensitive mutations in the following proteins as yeast is switched into a non-permissive temperature (5 each):

(a) SRP receptor

The rough ER will loss its roughness as ribosomes would not engage the translocon due to the lack of interaction of SRP with its receptor

(b) Sec 23

Class B mutant. Accumulation of ER due to the lack of vesicle budding

(c) T-snare in the cis golgi

Class C mutant. Accumulation of vesicles in the ER-Golgi transport

(d) ARF1

Class D. Transport from the trans Golgi reduced. Loss of lysosomal activity and endocytic activity.

(e) V-snare between trans-golgi and lisosome

Accumutlation of vesicles from the Golgiand loss of lisosomal activity

Name_____ SID _____

Q9 –- During the process of LDL endocytosys,

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

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

b) How are clathrin and LDL-receptor recycled? (10)

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

The receptors are sorted in the early endosome into vesicles and transported back to the plasma membrane.

Name_____ SID _____

Q10 – In the response of a liver cell to glucagon,

(a) How is the signal amplified? (15)

1- While the glucagon 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, like the dephosphorylation of glycogen synthase or the activation of transcription factors are OK, but do not add more points)

(b) How is the signal terminated (15)

1 - After the glucagon 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.

(c) Muscle cells do not contain glucagons receptors but contain epinephrine receptors that give rise to a very similar response in glycogen metabolism. Which signaling pathway in muscle cells antagonize the epinephrine pathway and results in glycogen synthesis? (10)

The insulin pathway that starts with the insulin receptor.

Name_____ SID _____

Q 11 - **For each DNA polymerase listed below (10 pts each), indicate each of (i) through (iv):**
(i) **Cellular function.** If there is more than one correct answer, choose ONE answer and then address the following parts of the question (ii- iv) according to the answer listedin (i). Note that "replication" or "repair" is not sufficiently specific; please specify a type of replication or repair.
(ii) **DNA synthesis processivity.** Choose from low (<5 nt), medium, or high (>500 nt).
(iii) **Nuclease activity (or activities) and their function(s)** that were discussed in class as part of the same polypeptide harboring the polymerase active site.
(iv) A **direct interaction with another protein** that recruits polymerase to template, IF any were described in class (answer for at least one polymerase is "none").

A. Polymerase I

(i)

(ii)

(iii)

(iv)

B. Polymerase III

(i)

(ii)

(iii)

(iv)

C. Polymerase V

Name_____ SID _____

(i)

(ii)

(iii)

(iv)

D. Terminal deoxynucleotidyl transferase

(i)

(ii)

(iii)

(iv)

E. Polymerase alpha

(i)

(ii)

(iii)

(iv)

Q12 - As you know, chromatin is a highly dynamic template that the transcriptional machinery of eukaryotic organisms must contend with in order to execute temporal

Name_____ SID _____

and spatially regulated gene expression. In short, different regions and segments of chromosome contain differently marked chromatin and these chemical "marks" in turn can have a profound impact on the activity of genes embedded in these differentially modified chromatin domains.

(10 pts.)

A. What are 3 common covalent "marks" that are frequently associated with specific chromatin regions? What feature of chromatin (i.e. nucleosomes) is the typical site for such chemical changes? How are these types of chemical "marks" thought to alter the activity of chromatin?

(10 pts.)

B. Each of these chemical marks is catalyzed by specific enzymes – what are the 3 types of enzymes involved? What is the chemical moiety that is transferred to chromatin by each of these different enzymes?

(10 pts.)

Name_____ SID _____

C. One of the key findings about chromatin modification is that individual genes and gene region display different patterns of modification depending on cell type, developmental stage, environmental cues, etc. What technique has allowed researchers to map specific chromatin "marks" along entire genomes of specific cell types? Briefly explain what critical reagent is needed for you to carry out this technique of mapping specific chemical modifications in a given cell type genome.

(10 pts.)

D. In addition to enzymes that covalently modify chromatin, another class of regulatory factor also plays a big role in "opening up" chromatin for transcription and gene expression. What is this other class of chromatin regulators and how do they work?

(10 pts.)

Name_____ SID _____

E. Like the chromatin modifiers, this other class of regulatory factors also functions in a gene and cell type specific manner. What method/experiment would you perform to show that one of these other classes of chromatin regulatory factors is specifically associated with an actively transcribed gene?