MCB110
FINAL

Dec 13, 2007

_____.
Your name and student ID

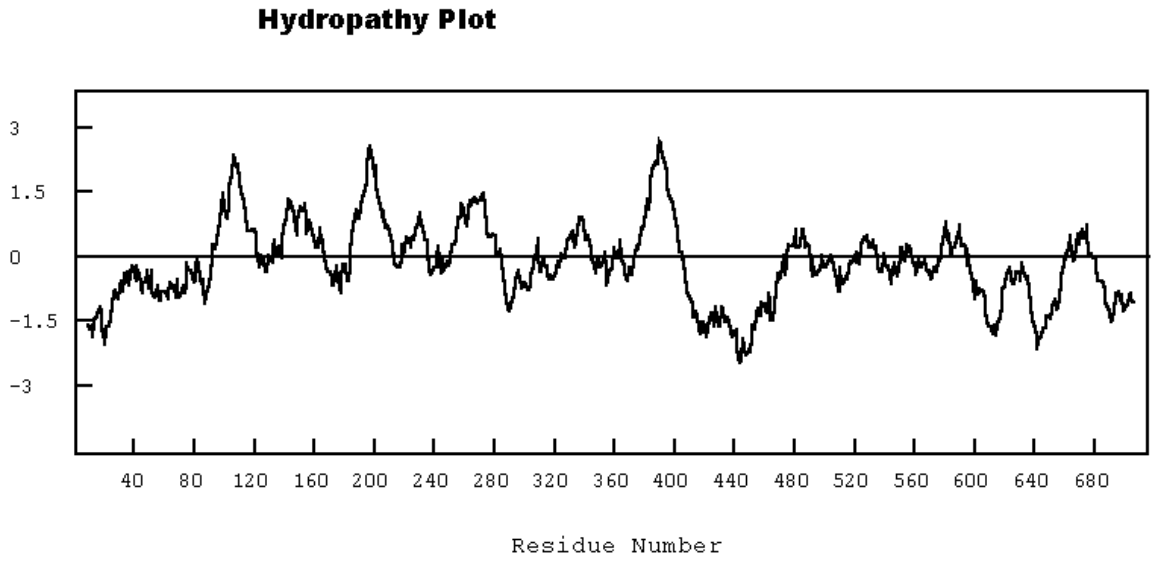
QUESTION	POINTS
1 (15 points)	
2 (15 points)	
3 (15 points)	
4 (20 points)	
5 (25 points)	
6 (20 points)	
7 (20 points)	
8 (20 points)	
9 (25 points)	
10 (25 points)	
11 (6 points)	
12 (10 points)	
13 (34 points)	
14 (50 points)	

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 - – Using the hydropathy plot below, do you believe the protein under study is an integral membrane protein? Why? (5 pts.) What can you predict from the plot in terms of secondary structure and why? (10 pts)



The plot indicates that this is an integral membrane protein with a number of transmembrane helical segments, indicated by stretches of 20-25 amino acids with high hydropathy index. Three such segments are very obvious at around residue 100, 200 and 390, with additional ones at about 150, 230, 270 and 340 (this could be a seven helix transmembrane, G-protein coupled receptor – 5 extra points for this).

Because 3 transmembrane helices are much more certain than the other 4, I would not penalize very severely those that just mention 3. The ideal answer will mention that 4 are likely but not clear.

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Q2 – What methodology would you use to determine the if a GPI-anchored protein that has been expressed as a GFP fusion is able to diffuse on the membrane? (5 pts) Describe concisely how the experiment will be carried out, and the possible outcomes. (10 pts)

FRAP - A defined region on the plasma membrane would be irradiated with a strong laser to photo- bleach the GFP. The intensity in this area as a function of time will then be measured. The plot of this intensity will start close to zero and then will increase until it reached a steady state plateau if the protein can diffuse (where how fast the recovery is obtains carries information on the diffusion coefficient), while it will remain close to zero if the protein cannot diffuse.

Q3 – The Ca2+ concentration in the cytosol is about 10^5 times lower than in the endoplasmic reticulum. What is the initial free energy of movement of Ca^{+2} through an IP3-gated channel? In your calculation you can ignore the term concerning voltage potential. Can you explain why? (15 pts.) (2.3 RT = 1.4 kcal/mol at 25 ˚C)

$\Delta G = 2.3\ RT\ [Cc]/[Cer] = 1.4 \times (-5) = -7$ kcal/mol

It makes sense to ignore the voltage component as the concentration gradient is so high and there is no significant voltage across the ER membrane.

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Q4 - Can you describe three main differences between the molecular mechanisms of a glucose facilitative transporter (or uniporter) and the glucose-Na co-transporter (10 pts) What is the role of these two transporters in the movement of glucose across an intestinal brush border cell? (10 pts)

1- the uniporter moves glucose down its concentration gradient, the symporter moves it against
2 – the uniporter does not require the presence of a Na gradient
3 – the uniporter can move glucose in whichever direction is defined by the gradient, while the symporter works only in one direction.

(I did not agree with Seemay on her objection, as I think that 1 and 2 are distinct and the students should point both out)

The simporter brings glucose from the lumen of the intestine into the cell, creating a large concentration gradient of glucose. The uniporter lets glucose out of the cell and into the blood stream, in this case down a concentration gradient.

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Q5 – Describe the events leading to the depolarization of a postsynaptic cell from the moment the action potential reaches the axon terminal of the presynaptic cell (15 pts). What is an important process that needs to be carried out at the synaptic cleft in order to reset the system and in which two ways can it be achieved (clue – cocaine interferes with this process)? (10 pts)

Voltage-gated calcium channels are open letting ca into de cell. This leads to fusion of vesicles loaded with neurotransmitter to the presynaptic plasma membrane and the dispersion of the neurotransmitter into the synaptic cleft. The neurotransmitter binds to ligand-gated Na channels in the postsynaptic cell, opening the channel and leading to the entrance of Na into the cell and thus to its depolarization.

The neurotransmitter needs to be cleared from the synapse. This is done either by breaking it down or by reuptake into the presynaptic cell via endocitosys.

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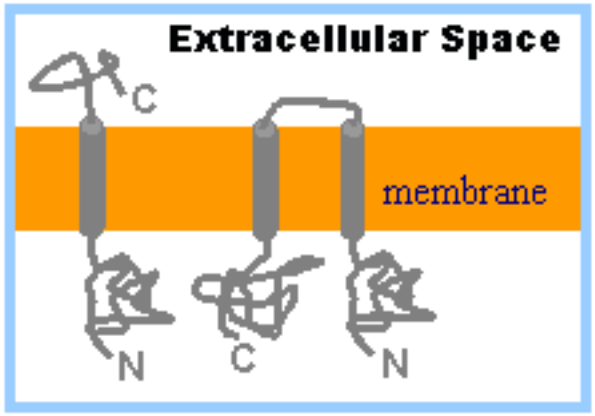
Q6 – For the two integral membrane proteins depicted in the figure, indicate the succession of signal sequence, signal-anchor sequences and/or stop-transfer sequences that would give rise to their topology in the plasma membrane (20 pts).



N-terminus …. SAII …. C-terminus

N-terminus …. SAII …. STA …. C-terminus

(Concerning Seemay comments, this is actually the only possibility that I can see. In both cases there is a big cytosolic N-terminus domain, and the ribosome will need a signal sequence to be bound by the SRP and taken to the ER (so in both cases there is an initial SAII – the +++ are OK if they include them in the right place –in front of the SAII, not behind!!!–, but if they do not mention them it is totally fine). For the second protein a STA then has to follow in order to define a second transmembrane region and then allow for the rest of the protein to be produced back in the cytosol).
Notice the orientation!!! The big domains are the ones produced in the cytosol, the top ones are the one going into the ER, as they are the ones that finish up outside of the cell. The reason this question has that many points is because it puts together two different concepts, the topology when engaging the ER, and the fact that this is maintained all the way into the plasma membrane. This is a question for the A an A+, I suppose.

Name_____ SID _____

Q7 – The structure of the co-translationally translocating ribosome bound to both the SRP and the translocon has been described. By which methodology (10 pts). How did this work indicate that the ribosome is never bound simultaneously to the SRP and the translocon (10 pts).

By electron microscopy and 3D reconstruction.
Because the surface of the ribosome interacting with the SRP overlaps with that interacting with the translocon.

Q8 – In the secretory pathway, at which point between the process of vesicle budding from the original membrane to fusion with the target membrane is GTP hydrolyzed and with which purpose? (5 pts) What would be the effect of a mutation that inhibits hydrolysis of GTP in the protein involved? (5 pts) What molecular process occurring after vesicle targeting and fusion to the target membrane requires energy input in the form of ATP hydrolysis? (5 pts) Which protein carries out this process (5 pts).

GTP is hydrolyzed by the G protein in the coat (Sar1 or Arf1) in order for the coat to breakdown.
Such mutation will lead to the accumulation of vesicles that cannot fusion to their target membrane.
The breakdown of the snare complex for recycling.
This is done by NSF.

Name_____ SID _____

Q9 – What molecular events lead to cAMP production in muscle cells following binding of epinephrine to its receptor? (5 pts) How is PKA consequently activated and how does it result in glycogen breakdown? (10 pts) What two molecular processes are actively required to shut down glycogen breakdown after epinephrine disappears from the blood stream? (10 pts). (No points will be granted for information on epinephrine response not related to these specific questions!)

Epinephrine binds to its g-protein coupled receptor. This results in a conformational change in the receptor that increases its affinity for the alpha subunit of a stimulatory trimeric G-protein and leads to the release of GDP from it. GTP then binds to the site, activating the G protein and leading to the separation of the alpha subunit from the beta-gamma complex. The active, GTP-bound alpha subunits binds and activates adenylyl cyclase.

cAMP binds to the regulatory subunit of PKA releasing the catalytic subunit that now becomes active as a kinase. PKA then phosphorylates phosphorylase kinase (PK), activating it. In turn phosphorylase kinases phosphorylates and activates phosphorylase (P), which then catalitically breaks down glycogen.

The breakdown of cAMP by phosphodiesterase, and the dephosphorylation of PK and P by phosphatase1.

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Q10 – Specific cellular localization is essential in many signaling pathways. How is PKB (protein kinase B) localized to the membrane following insulin signaling (5 pts) and how is this localization important for further events down this pathway, such as GLUT4 mobilization? (5 pts). Another example has to do with the activation of the membrane-linked G-protein Ras. What signaling elements are sequentially recruited to the plasma membrane resulting in Ras activation, irrespective of the type of ligand/receptor involved in the initiation of the signaling event (5 pts)? Describe how the activation of Ras ultimately results in cell proliferation (10 pts).

PKB needs to be localized to the plasma membrane where it will get phosphorylated and activated. This happens by its interaction with PIP2, which itself is generated when the PI-3 kinase is recruit via its SH2 domain to phosphotyrosine residues in IRS adapters that bind the phosphorylated insulin receptors.

The phosphorylated PKB is then active in down stream steps.

Phoasphotyrosine residues recruit the adaptor Grb2, which then recuruits to the membrane SOS, the Ras GEF, resulting in GTP-bound Ras. Active Ras binds and activates Raf (or MSAPKKK), which activates the MAP kinase cascade that ultimately results in the activation of transcription factors regulating the transcription of genes required for cell proliferation.

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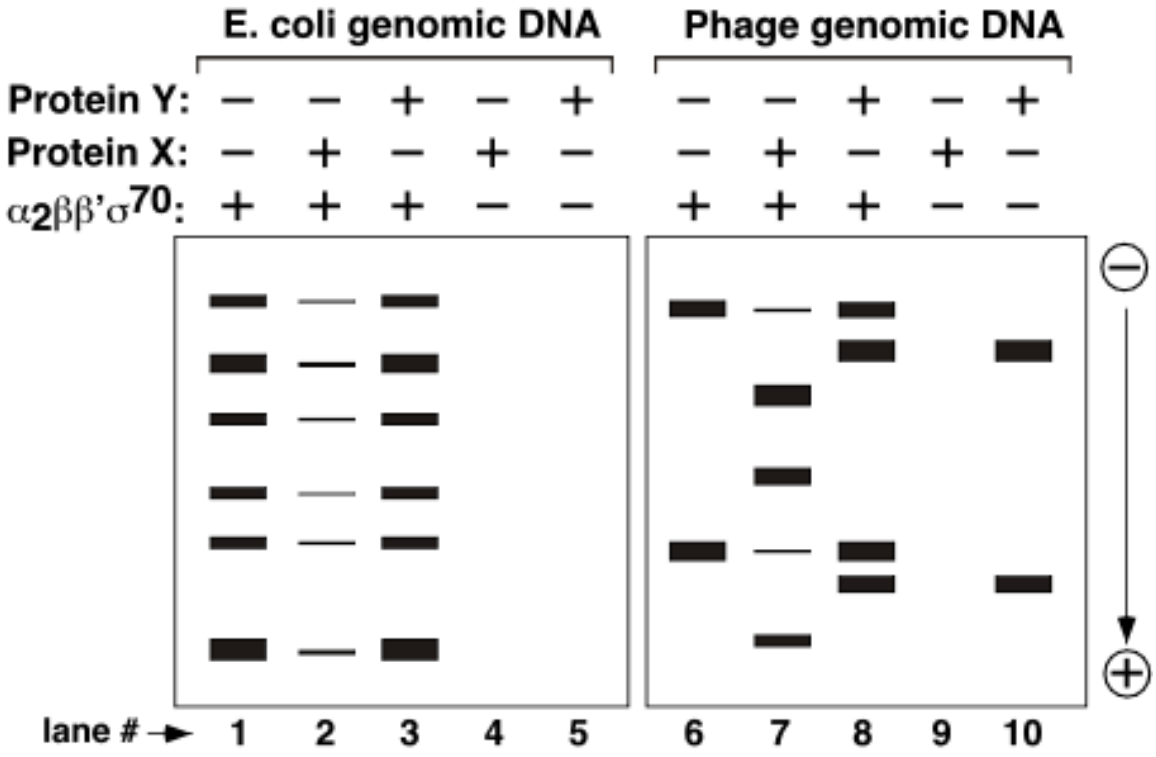
Q11 - In the lab where you are doing your thesis research, two proteins, X and Y, that appear to have transcriptional activities have recently been identified and purified from bacterial phage T101. For your thesis project, you want to further investigate the functions of X and Y by performing an in vitro transcription assay. This assay, which is already established in the lab, supports efficient transcription when pure E. coli RNA polymerase holoenzyme $\alpha 2\beta\beta'\sigma 70$ is incubated with a 5-kb E. coli genomic DNA fragment (as a template) and ^{32}P-labeled ribonucleotides (e.g. see lane 1 of the figure below). Now, you are adding purified X or Y at a 10:1 molar ratio over the E. coli holoenzyme into transcription reactions containing the above-mentioned E. coli genomic DNA fragment. The autoradiogram of the transcription gel is shown below (lanes 1-5). The various bands represent RNA transcripts produced from different promoters present on the E. coli genomic DNA, and the intensity (or thickness) of the bands correlate with the levels of transcription from these promoters. To further investigate the roles of X and Y in transcription, you carry out a similar set of reactions using instead the phage T101 genomic DNA as a template and the result of this experiment is shown in lanes 6-10 (again a 10:1 ratio of X or Y over holoenzyme in reactions #7 and #8).



(1) Using one sentence, please describe what kinds of transcription factors X and Y are or what the likely roles of X and Y are in transcription? (Please be very specific and don't just state the obvious, such as Y is a phage-encoded transcription factor and it plays no role in transcription from the E. coli genomic DNA and a positive role in transcription from the phage DNA) (**10 points**)

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(2) Your experiments indicate that there are three types of promoters present in the phage T101 genome. What are they and how are they transcribed? **(12 points)**

(3) In lane 7, two bands with the same mobility as in lane 6 show markedly decreased intensity. Meanwhile, three new strong bands are showing up in this lane compared to lane 6? What causes these differences? What is responsible for the difference seen between lanes 6 and 8? **(14 points)**

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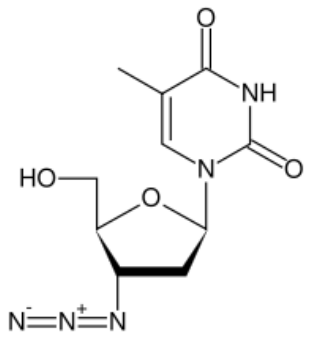
(4) What could be the molecular mechanism by which X affects transcription in lane 2 in comparison with lane 1? Please design an experiment to prove your stated mechanism, assuming that you have access to all the necessary reagents. **(14 points)**

Q12 - Describe how the 3' -> 5' exonuclease domain of DNA polymerase I contributes to the high fidelity of the enzyme. (How does the enzyme make product if the exonuclease domain degrades it?) (**6 pts.**)

Name_____ SID _____

Q13 A - Here is the chemical structure of the HIV therapeutic azidothymidine (AZT). How do you think this drug inhibits replication of the HIV genome? (**5 pts.**)	

Q13B - AZT is an example of a prodrug, which is an inactive compound that is transformed into an active drug *in vivo*. What transformations of AZT are required for it to work in the way you proposed in A? (**5 pts.**)

Q14A - Single-stranded oligonucleotides as short as 30 bases, when introduced into bacteria or yeast, can cause mutations encoded in the oligonucleotide. Propose <u>two possible mechanisms</u> by which the mutation encoded in the oligonucleotide can be transferred into the bacterial or yeast chromosome. (**8 pts.**)

1.

2.

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Q14B - The oligonucleotide-induced mutations in *E. coli* did not require active RecA and mutagenesis was much more efficient when the mutation was in an oligonucleotide complementary to the lagging strand. (Oligonucleotides complementary to the leading strand were not efficient.) Which mechanism of mutagenesis you proposed in A do these results support? Briefly explain why these two results support our answer in A. (**12 pts.**)

Q14C - In wild-type *E. coli*, the frequency of base-substitution mutations induced by an oligonucleotide nearly complementary to the lagging strand was ~0.06%. Mutations in one of the DNA repair pathways increased the mutation frequency 100-fold to 6%. Which repair pathway do you think is involved in correcting the oligonucleotide-induced base substitutions? Briefly explain how this pathway corrects substitution mutations and not the wild-type sequence. (**8 pts.**)

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Q14D - What is your favorite use for synthetic oligonucleotides in molecular biology? Briefly explain what this technique enables people to do and how the technique works, emphasizing the role(s) of the oligonucleotide(s) (**6 pts.**)