Name _____

7.06 Cell Biology
Exam #1

This is a closed book exam. You are allowed only two pages of notes, but not computers or any other types of electronic devices.

Please write your answers to the questions in pen (not pencil) in the space provided.

Please write only on the FRONT SIDE of each sheet.

Be sure to put your first and last name on each page in case they become separated.

There are **9** pages to the exam. Make sure that you have a complete copy.

Remember that we will photocopy all of the exams before returning them to you.

Good luck.

Question 1. 12 points

Question 2. 12 points

Question 3. 14 points

Question 4. 10 points

Question 5. 14 points

Question 6. 38 points

Total. 100 points

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Question 1 (12 points)
Green Fluorescent Protein (GFP) is a powerful tool for cell biology. Each of the following is a real variation on GFP that someone has developed. You have generated fusions between these GFP variants and a protein (or proteins) of interest. Briefly describe the nature of the protein's interactions, localization, or other aspects of its properties or behavior that you would be able to test using each variant.

A. In addition to traditional GFP, you have a version of GFP in which the emission and excitation spectra are altered to cause this to appear as a Red Fluorescent Protein. (3 pts)

This will allow you to observe the relative behavior and localization of two different proteins (one fused to GFP and the other to RFP). For example, you could detect their co-localization or use this to simultaneously observe two different cellular structures.

B. A version of GFP in which a pulse of light at a specific wavelength will convert its emission and excitation spectra from green to red (photoconvertible). (3 pts)

This will allow you to detect the dynamic behavior of a pool of a protein. You could use this similarly to photoactivatable GFP to "turn on" the fluorescence of a localized pool. However, in this case, you could also visualize the relative behavior of the light activated population (red) compared to the rest of the protein (green).

C. A version of GFP that possesses a very slow maturation time for achieving its fluorescence. The protein is produced and folded rapidly, but it takes >20 hours for it to display fluorescent behavior. (3 pts)

Using this fusion, you would only detect proteins that are older (more recently synthesized proteins would be invisible). In this way, you could detect the relative behavior of older proteins, for example to see if they display a distinct localization. If you didn't detect fluorescence at all, this would indicate that the protein is less stable or degraded.

D. A version of GFP in which the coding sequence is split into two separate proteins. Neither portion is fluorescence on its own, and they also have low affinity for each other such that they will not associate when expressed at normal levels. However, when the two halves are brought together artificially in close proximity (within 10 Å), they will reform GFP and display fluorescence. (3 pts)

This will allow you to detect when two proteins are in close molecular proximity. This is more than simply co-localization, as based on the defraction limit of light, this would only tell you when two proteins are within ~200 nm. Therefore, this split GFP tool would tell you when two proteins are bound to each other, or allow you to map about the close interactions within a larger molecular structure (such as the nuclear pore).

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Question 2 (12 points)
In class, we discussed various ways that molecules can cross a membrane. These include channels, transporters, and the nuclear pore, both of which effectively generate a specific and regulated "hole" in the membrane. One key property of both pumps/channels/transporters and the nuclear pore is the directionality of transport. *Below, briefly explain your answers in each case*.

A. Provide an example of a situation in which a **pump, channel or transporter** would allow a molecule to pass based on the relative concentrations of that molecule on either side of the membrane. (3 pts)

This would be the case for a standard channel (either a gated or ungated channel). For example, the K+ channel that relieves the gradient of ions generated by other channels, or the voltage gated Na+ channel that opens in response to changes in charge, but still lets Na+ travel down the gradient.

B. Under what conditions (if any) would the **nuclear pore** allow a protein to pass based on the relative concentrations of that molecule on either side of the membrane. (3 pts)

This is the case for a protein less than 40 kDa that DOES NOT contain an NLS or NES. In this case, the protein can pass (diffuse) across the pore, but does so without a specific directionality (based on the relative concentrations of that protein).

C. Provide an example of a situation in which a **pump, channel or transporter** would allow a molecule to pass such that the molecule displayed a highly increased concentration following its transport. (3 pts)

In this case, the pump or transporter needs to rely either on the energy input (ATP) or a different gradient (for example, for an symporter) to ensure the directional transport of the molecule up a gradient. This is true for the K+/Na+ anti-porter, or the Na+/glucose symporter.

D. Under what conditions (if any) would the **nuclear pore** allow a protein to pass such that the molecule displayed a highly increased concentration following its transport. (3 pts)

This will be the case for proteins (of any size) that contain an NLS or NES. In this case, the Ran-GTP gradient will provide directionality to import/export a protein up a gradient of its concentration.

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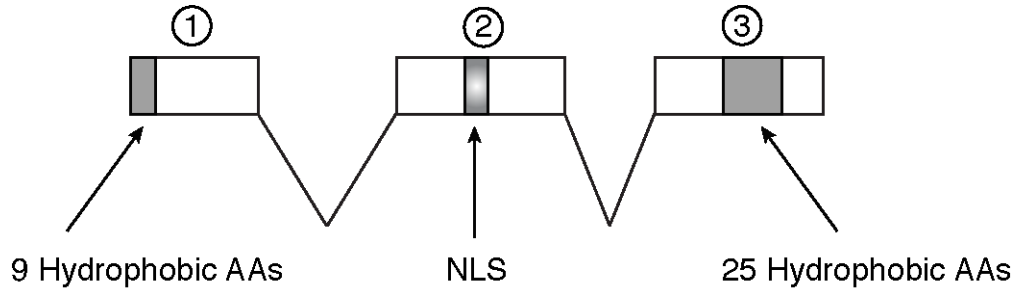
Question 3 (14 points)
You are working on a protein that is alternatively spliced. The diagram below shows the three exons (and spliced out introns) for the full-length mRNA (with exons 1, 2, and 3). However, there are multiple versions of this protein that exist with different combinations of these exons. For isoforms lacking exon 1, there are internal start sites that will allow the protein to be produced.



For each of the spliced version listed below, indicate the likely localization of the protein. For membrane proteins, also indicate whether these are Type I or Type II proteins. (2 pts each)

A. Exon 1

Secreted

B. Exon 2

Nuclear

C. Exon 3

Plasma membrane (transmembrane, Type II)

D. Exon 1 + 2

Secreted

E. Exon 2 + 3

Inner nuclear membrane (transmembrane)

F. Exon 1 + 3

Plasma membrane (transmembrane, Type I)

G. Exon 1 + 2 + 3

Plasma membrane (transmembrane, Type I)

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Question 4 (10 points)
You are using Fluorescence Recovery After Photobleaching (FRAP) experiments to test the turnover of different membrane proteins.

A. You first test the turnover of a plasma membrane-localized transmembrane protein (in which you have added a GFP tag to its C-terminus) by bleaching a portion of the plasma membrane. For the wild type protein, you find the t½ for recovery is 3 minutes. You next remove the first 10 amino acids of the proteins (leaving the start codon), which contain the sequence "RLIVLMIIVL". It still localizes to the plasma membrane, but you find instead that its t½ for recovery is 20 seconds. Provide a model that explains the change in fluorescence recovery and the effect of the N-terminal deletion on the properties and behavior of the protein. (4 pts)

The amino acids that have been removed correspond to a 9 amino acid N-terminal hydrophobic signal sequence. Removing these residues will change this protein from a type I transmembrane protein to a type II protein, reversing its orientation in the plasma membrane. The FRAP data indicates that this switch in orientation makes the protein much more dynamic. This suggests that it is now not able to interact with an interaction partner that would perturb its mobility and diffusion. For example, due to the swap, the formally extracellular domain is now internal and would not interact with a second extracellular protein or ligand.

B. You next test the recovery of a protein that is localized to the ER lumen. You develop a laser bleaching protocol that allows you to specifically bleach the entire ER. Following the photobleaching, the t½ for recovery for ER fluorescence is 45 minutes.

• If you were to mutate (eliminate) the KDEL receptor, would the turnover of your protein be faster, slower, or unchanged? Assume that the KDEL receptor is permanently inactivated at the precise time of your photobleaching. ***Briefly*** explain your reasoning. (3 pts)

This would likely be slower. In this case, fluorescence recovers by importing newly synthesized proteins into the ER. However, for this ER resident protein, this will "leak"/escape from the ER at some frequency, to be returned to the ER by the KDEL receptor (and vesicle trafficking). In the absence of this recovery mechanism, fluorescence accumulation (and recovery) will be slower).

• If you were to mutate (eliminate) Sec61, would the turnover of your protein be faster, slower, or unchanged? Assume that Sec61 is permanently inactivated at the precise time of your photobleaching. ***Briefly*** explain your reasoning. (3 pts)

This will be much slower to the point that recovery is unlikely to happen at all. To get new fluorescence, newly synthesized protein needs to be imported into the ER through the Sec61 channel. If the channel is non-functional, no new protein will be imported and there can be no ER fluorescence.

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Question 5 (14 points)
You are studying viral infection of mammalian cells, focusing on RNA viruses. For these viruses to infect a host cell and replicate, a key step is for the viral RNA to enter the host cytoplasm. An intact viral particle contains a protein "capsid" (shell) that is approximately 100 nm in diameter, additional associated proteins, as well as the viral RNA.

A. What types of molecules are able to cross the plasma membrane on their own? (2 pts)

They need to have hydrophobic character such that they can cross the fatty acid portion of the lipid chains (examples are fine, but not needed).

You are testing two different viruses (Virus A and Virus B). In each case, you infect the host cell with the virus. After a brief incubation, you then treat cells with the protease trypsin, wash the cells, and then lyse the cells with detergent. For both viruses, you are able to detect the viral RNA in the cell lysate following these steps.

B. For Virus A, following these steps, you are able to detect the viral capsid proteins by Western blot of the cell extract. Propose a model by which the virus and the viral RNA is able to enter the host cell. (4 pts)

By endocytosis. The entire viral particle including the capsid and the RNA is internalized by the cell. It then goes to the endosome, but will now need a mechanism to release from that membrane compartment and enter the cytoplasm (it's not necessary to specify how this occurs).

C. For this virus, you find that viral proteins are required for host cell entry and the cell type specificity of its infection. Explain why this is the case. (4 pts)

Endocytosis (particularly clathrin-mediated endocytosis), requires that a receptor on the cell surface interact with a cargo to drive its internalization. Thus, the virus needs to have proteins that can associate with receptors on a cell, and each cell will express a different range of receptors (giving this cell type specificity).

D. For Virus B, following similar steps, you are able to detect the viral RNA, but you are UNABLE to detect any viral proteins by Western blot of the cell extract. However, despite the fact that you do not detect viral proteins in Western blots of cell extracts, you find that entry of the viral RNA requires the function of the capsid-associated viral proteins. Propose a model by which the viral RNA is able to enter into the host cell. (4 pts)

In this case, the virus associates with the cell and injects the RNA into the host cell, leaving the capsid on the cell surface, but not entering itself. For this to occur, the virus must form a channel or pore in the membrane that is sufficient to allow the RNA to pass through.

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Question 6 (38 points)
You are working to define the order of events during vesicle trafficking in the secretory pathway. For these experiments, you are working in a cell type that is highly secretory such that the majority of the newly synthesized proteins are destined for secretion from the cell.

You have developed a protocol for differential centrifugation that allows you to generate purified populations of nuclei, ER, Golgi, plasma membrane, and vesicles, as well as a cytoplasmic (membrane-free) fraction, and the extracellular media.

A. You have obtained a source of radioactive amino acids that you can "feed" to the cells. Following your differential centrifugation procedure, you detect can radioactivity in each fraction. Using this as a tool, describe how you could define the general order of the secretory pathway. At this stage, you do not have access to specific mutants. (4 pts)

You could do a pulse-chase experiment. In brief, at a set time point, you will feed the cells with the radioactive amino acids. You will then wash these away and feed with unlabeled amino acids. You will then take time points at regular intervals, process the cells (lysis and centriguation, etc.), and detect where the radioactivity exists. You should see the labeled amino acids move through the secretory pathway as the protein is synthesized, imported into the ER, trafficked to the Golgi, and then trafficked to the plasma membrane for secretion.

B. Is this assay a "population-based" assay, a "single-cell" assay, or "single-molecule" assay? ***Briefly*** explain your answer. (2 pts)

Population-based assay. You are looking at the average behavior of multiple cells growing in your dish using a biochemical approach.

C. You next isolate a series of mutants in this cell line. In each mutant below, where (if anywhere) would you see an ***accumulation*** of the labeled amino acids relative to control cells? Specify, cytoplasm, nuclei, ER, Golgi, vesicles, plasma membrane, or extracellular (i.e. secreted). Assume that these mutants act relatively fast (i.e., you are looking at primary defect, not secondary consequences). Unless otherwise indicated, assume that the mutant is a loss of function (i.e., you are looking at a temperature sensitive at the restrictive temperature).
(2 pts each)

• A Sar1 mutant in GTP trapped state (the GTPase responsible for ER to Golgi transport)

ER (if it is always in the GTP state, can't locally induce coat formation)
1 pt given for vesicles as the answer (can't undo coat formation)

• A Sar1 mutant in GDP trapped state

ER (can't trigger coat assembly to traffic out of the ER)

• BiP

Cytoplasm (can't import into the ER)

• SRP

Cytoplasm (can't import into the ER)

• Sec61

Cytoplasm (can't import into the ER)

• KDEL receptor

No accumulation (will still be secreted just fine).

• Plasma membrane t-snare

Vesicles

• v-snare for targeting to the plasma membrane

Vesicles

• The Ran GEF (also called RCC1)

No accumulation (will still be secreted just fine).

D. You have isolated three different mutants in the secretory pathway. Mutant A shows an accumulation of amino acids in the Golgi. Mutant B and Mutant C both show an accumulation of labeled amino acids in vesicles. However, in the double mutants, Mutant A + Mutant B displays an accumulation of labeled amino acids in vesicles whereas Mutant A + Mutant C displays an accumulation in the Golgi. What does these data suggest about the potential role of each protein and the order of these proteins within the secretory pathway? (6 pts)

Mutant B and C are both involved in trafficking steps related to vesicles (for example, by preventing the vesicle from fusing with its target), but the vesicles that where the amino acids are accumulating are different in each case (Mutant C is vesicles that have left Golgi, and Mutant B is vesicles before the Golgi – from the ER). Mutant A will prevent proteins from leaving the Golgi.

The order at which the protein products act is B → A → C.

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E. For each of the following cellular trafficking events, indicate whether this event is reversible or irreversible. In either case, indicate what ensures the directionality of this event. (2 pts each)

• Nuclear import

Reversible. Directionality is ensured by the Ran-GTP gradient, such that NLS containing proteins can associate with Importin in the cytoplasm and separate from Importin in the nucleus.

• Trafficking from the ER to the Golgi

Reversible. Proteins can traffic in both directions. COPI/Arf and COPII/Sar1 provide some specificity for these different trafficking events, but the primary thing controlling this directionality is what proteins are recognized for Golgi to ER trafficking (for example, if they contain a KDEL sequence).

• Insertion and translocation of a protein into the ER

Irreversible. Directionality is ensured by the BiP molecular chaperone and the fact that the protein folds once it is inside the ER such that it can't pass back through the Sec61 channel

• Vesicle coat formation

Reversible. The Arf and Sar1 GTPase provide the directionality for coat formation, with the GTP bound state allowing coat formation to occur and hydrolysis to the GDP bound state causing the coat to disassemble.