Name _____

7.06 Cell Biology
Exam #2

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.

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

There are **11** 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. 5 points

Question 2. 6 points

Question 3. 5 points

Question 4. 8 points

Question 5. 12 points

Question 6. 29 points

Question 7. 5 points

Question 8. 30 points

Total. 100 points

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Question 1 (5 points)
For the proteins listed below, circle each protein that plays an inhibitory role in its corresponding signal transduction pathway.

PTEN	Axin	SMAD	
PI3 Kinase	GSK3β	SnoN	I-KB
Presenilin	Notch	Ras	

Question 2 (6 points)

A. The bacteria that causes food poisoning, *Clostridium perfringens*, secretes a peptide toxin. This toxin can bind to some claudins, and when it does, the N terminus of the toxin inserts into the membrane, forms a hole in the membrane, and kills the cell.

Circle below the cell types that you predict will be vulnerable to killing by the toxin:

Kidney	Cardiac muscle
Bladder	Nerve cells
Skeletal muscle	Intestine

B. The smooth muscles that surround the digestive tract must contract in synchrony to generate the peristaltic movements that move food through the intestine. Calcium fluxes are important in muscle contraction. What type of cell-cell junction is likely to be critical in these smooth muscles? ***Briefly*** explain your reasoning.

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Question 3 (5 points)
You have identified two different signaling pathways (A and B). For pathway A, when you add the corresponding ligand, gene expression is rapidly activated (5 minutes). Then, when you remove the ligand, gene expression is rapidly eliminated (5 minutes). In contrast, for pathway B, ligand addition causes rapid pathway activation, but gene expression persists for >1 hour when the ligand is removed. When you inhibit protein translation, pathway B gene expression persists indefinitely after ligand removal (>4 hrs – the longest time point in your experiment). Inhibiting translation has no effect on pathway A.

Provide a model to explain the nature of and differences between these pathways, including the likely types of proteins involved.

Question 4 (8 points)
The RIII receptor in the TGFβ pathway plays an essential role in TGFβ signaling. Normally, the RIII receptor binds to TGFβ with an affinity of 100 nM (10^{-7} M).

A. You identify a mutant in the RIII receptor that instead binds to TGFβ with an affinity of 100 μM (10^{-4} M). What would be the effect of this mutation on TGFβ signaling? Be sure to answer considering the **specific functions of the RIII receptor** in this pathway. ***Briefly*** explain your answer.

B. You next identify a mutant in the RIII receptor that binds to TGFβ with an affinity of 1 femtomolar (10^{-15} M) – similar to the affinity of biotin and avidin. What would be the effect of this mutation on TGFβ signaling? Be sure to answer considering the **specific functions of the RIII receptor** in this pathway. ***Briefly*** explain your answer.

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Question 5 (12 points)

For a signal transduction pathway, a key criterion is that it can recognize a signal "input" and use this to affect downstream signaling. In cells, one important signal is the presence of mechanical force, either exerted on cells or produced intra-cellularly. For each of the situations below, **propose a model** for how force is connected to the production of a downstream signal.

A. Intracellular force is exerted at opposite ends of a ***substrate*** for a constitutively active kinase, pulling its ends apart. In this case, when the substrate is under tension (force), the phosphorylation of the substrate (and downstream signaling) **can** occur.

B. Intracellular force is exerted at opposite ends of a ***kinase*** (i.e., the N- and C-termini) pulling pulling its ends apart. In this case, when the kinase is under tension (force), downstream signaling **does not** occur.

C. The kinetochore is a multiprotein complex that connects DNA and microtubules. A key kinase localizes to the DNA, and its substrates associate with the microtubule. During mitosis, force stretches the proteins of the kinetochores so that the DNA and microtubule separate by more than 60 nm. Signaling occurs in the absence of force, but not in the presence of force.

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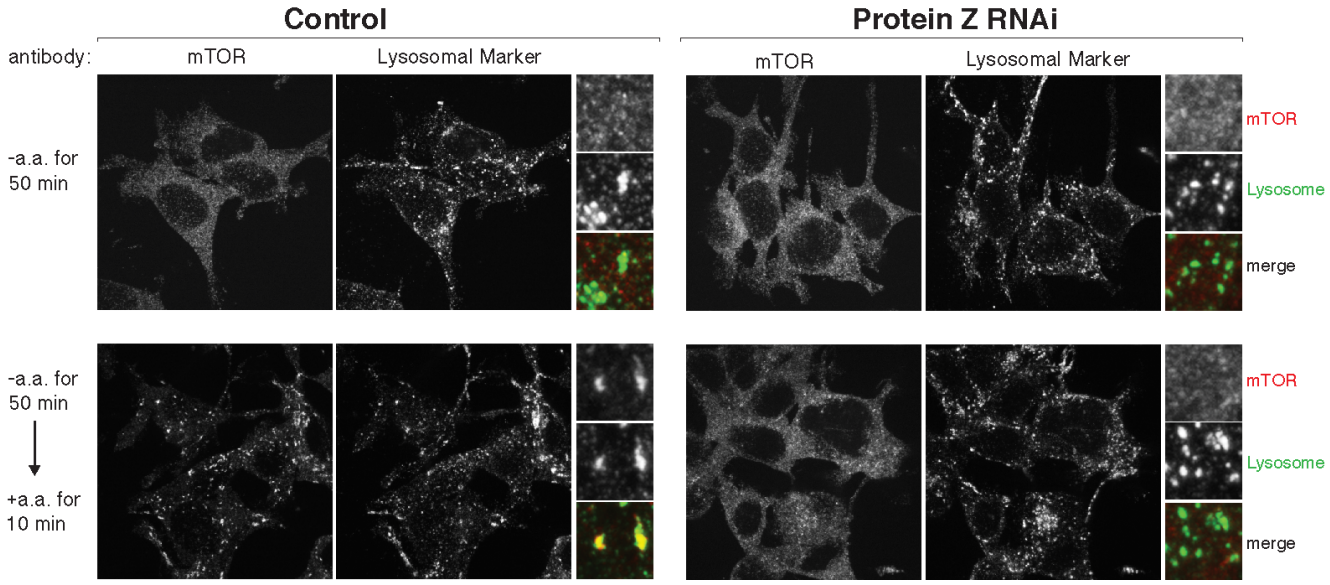
Question 6 (29 points)

The mTOR signal transduction pathway plays a key role in sensing a cell's environment to promote cell growth. In the presence of amino acids, the mTOR signal transduction pathway is activated to promote protein translation and cell growth. You are working to order this complex signal transduction pathway. In addition to mTOR itself, you have identified 4 key proteins (Protein X, Y, Z, and G).

You first examine mTOR localization. To do this, you starve the cells of amino acids by removing them from the media. You then add back amino acids (indicated as "a.a" in figures below) for a short period of time. In each case, you test mTOR localization using a specific antibody and compare this to the localization of a marker for the lysosome. For the images below, a "blow up" (increased magnification) is shown on the right to help evaluate co-localization.



A. What is the effect of amino acid treatment on mTOR localization? (2 points)

B. Based on the data shown above for cells depleted of Protein Z by RNAi, indicate whether this is likely to be playing a positive or negative (i.e., inhibitory) role in mTOR signaling in an unperturbed cell. ***Briefly*** explain your reasoning. (3 points)

C. Provide an example (pathway and specific step) from the key signal transduction pathways that we discussed in class in which this type of cellular localization-based assay would help for assessing pathway activation. (3 points)

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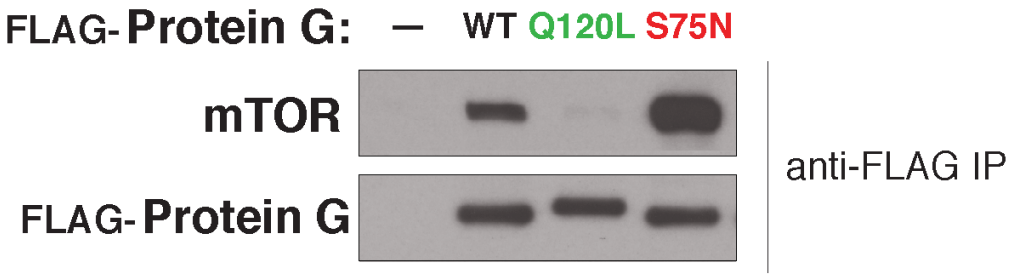
You next test for physical interactions between the proteins in the mTOR pathway. To do this, you express a FLAG (epitope) tagged version of Protein G (a G-protein - GTP-binding protein), and immuno-precipitate (IP) the protein using anti-FLAG antibodies to isolate associated proteins. You then run the isolated proteins on an SDS-PAGE gel, and perform a Western blot using antibodies against Protein G and mTOR itself. In addition to expressing an empty vector control (-) and a wild type (WT) version of Protein G, you also express a Q120L mutant that traps this protein in the GTP bound state and a S75N mutant that traps this protein in the GDP bound state.



D. Based on these data, what is the nature of the interaction between Protein G and mTOR? ***Briefly*** explain your answer. (4 points)

E. Provide an example (pathway and specific step) from the key signal transduction pathways that we discussed in class in which this type of immunoprecipitation-based assay would help for assessing pathway activation. (3 points)

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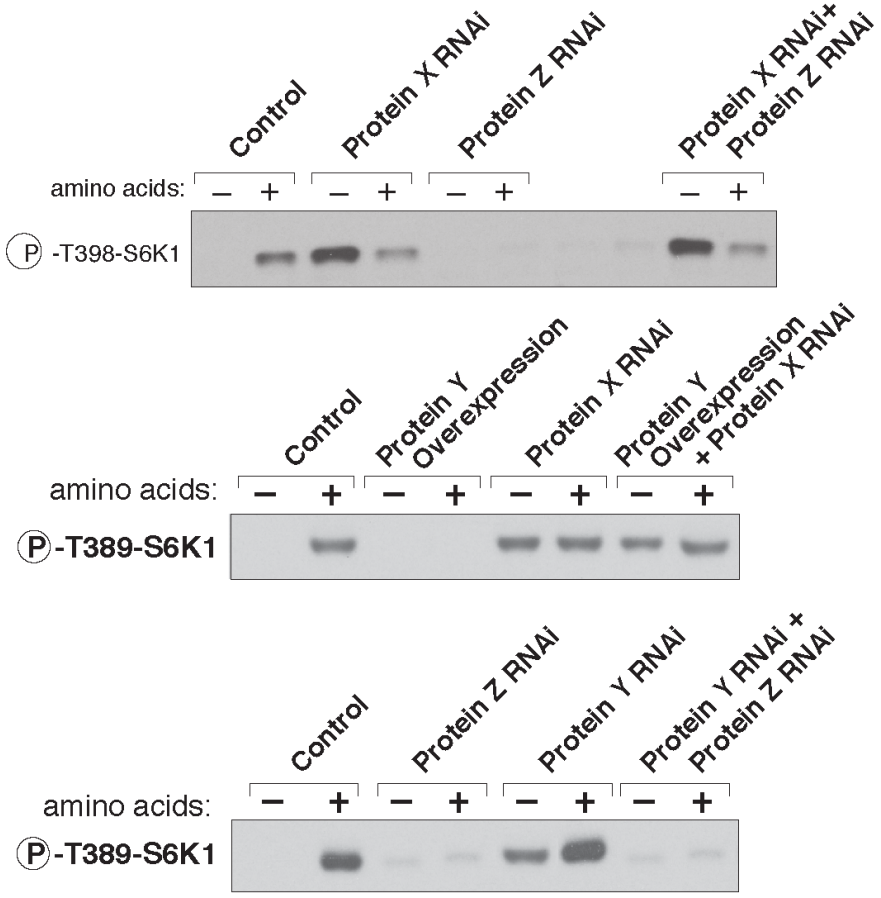
Finally, you test the activation of the mTOR signaling pathway in the presence and absence of amino acids by analyzing the phosphorylation of a key downstream substrate (S6K1) using a phosphorylation-specific antibody against a key residue in this protein.

The Western blots below test S6K1 phosphorylation in the absence of amino acids (starvation) or following amino acid treatment. Each blot represents a set of pairwise experiments with individual treatments (RNAi or protein overexpression) and the double treatment.



F. Based on these data, for each pair of proteins indicate the likely relationship between these proteins using → and ⊥ symbols. Be sure to connect these to amino acid treatment and S6K1 phosphorylation. (9 points)

Protein X and Protein Z -

Amino acids S6K1 Phosphorylation

Protein X and Protein Y -

Amino acids S6K1 Phosphorylation

Protein Y and Protein Z -

Amino acids S6K1 Phosphorylation

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Directly inhibiting mTOR activity prevents S6K1 phosphorylation in both the presence and absence of amino acids. Inhibiting mTOR also prevents S6K1 phosphorylation in each of the perturbations described above

G. Based on all of the data provided in this question, propose the most likely order of these proteins within the mTOR signal transduction pathway (with using → and ⊥ symbols). Be sure to include each of these proteins (Protein X, Y, Z) and mTOR itself, as well as amino acids and S6K1 phosphorylation. If you are unable to precisely order a given protein or provide its relationship (→ or ⊥), indicate why this is the case. ***Don't include Protein G in this ordering***. (5 points)

Amino acids

S6K1 Phosphorylation

Question 7 (5 points)
For ordering a signal transduction pathway, if your only readout was pathway activation (on/off), would you be able to order a pathway using only **loss-of-function** mutations? Explain why or why not? Be sure to consider **diverse** signal transduction pathways.

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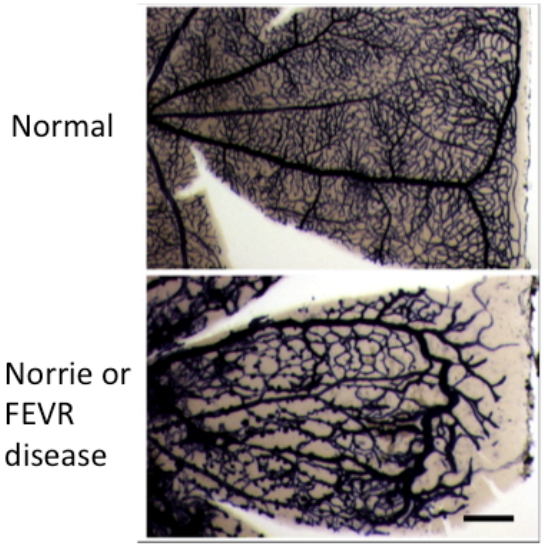
Question 8 (30 points)

Signal transduction pathways can be used in a variety of developmental contexts. This problem asks you to evaluate a new use for one signal transduction pathway.

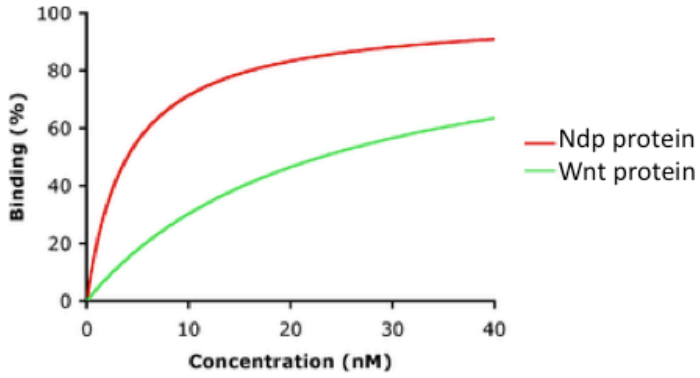
There are hereditary forms of blindness in humans that result from a failure to vascularize the retina. In these diseases (termed "Norrie" and "familial exudative vitreoretinopathy (FEVR)"), both arteries and veins are missing in the retina (see below).

Vascularization in the retina



The Norrie gene has been cloned and was found to encode a new protein, **Ndp**. One of the genes affected in FEVR disease corresponds to one of the several Frizzled genes present in mammals, **Fz4**. Wnt is not expressed in the developing retina.

A. (4 points) You first conduct an experiment to express Fz4 in tissue culture cells. You then add either labeled Ndp or labeled Wnt to these Fz4-expressing cells at different concentrations and measure the binding of these molecules to the cells.



What two conclusions do you draw from these results?

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B. (6 points) If you wanted to test the effect of Ndp on signaling through Fz4 using this cell culture system, assuming that Fz4 signaling is similar to other Fz receptors, what would you assay (i.e., measure)?

What membrane protein would have to be expressed by these cells?

What intracellular protein would have to be expressed by these cells?

C. (2 points) You next decide to develop a mouse model to study the role of these proteins (which are conserved in mice) in retinal vascularization. You make a null mutant allele of Fz4 and find that homozygous mice are blind with retinal vascularization defects. You also obtain mice heterozygous for a null allele of β-catenin.

Would you predict that you would be able to obtain homozygous null β-catenin mutant mice? ***Briefly*** explain your reasoning.

D. (4 points) Mice that are heterozygotes for Fz4 (Fz4+/-) or β-catenin (β-catenin+/-) have normal sight. However, when both genes are heterozygous in the same mouse (Fz4+/-; β-catenin+/-), the mice are blind.

What two conclusions can you make based on these data?

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E. (6 points) It is possible to generate mosaic mice in which a subset of cells are homozygous mutant clones, but the rest of the animal is heterozygous. This is done by recombining out the wild-type copy of the gene in a specific tissue. Mutant clones can be made in the entire retina, in just the cells that produce the blood vessels, or in the glia cells that lie adjacent to the blood vessels. You make mosaic mice that are mutant for Ndp or Fz in the retina, retinal blood vessels, or retinal glia and test the mice for blindness.

Entire Retina	Blood Vessels	Glia	Phenotype
Ndp-/-	--	--	Blind
Fz4-/-	--	--	Blind
Ndp+/-	--	--	Normal
Fz4+/-	--	--	Normal
--	Ndp-/-	Ndp+/-	Normal
--	Ndp+/-	Ndp-/-	Blind
--	Fz4-/-	Fz4+/-	Blind
--	Fz4+/-	Fz4-/-	Normal

What do you conclude about the role of Ndp and Fz4 and the pathway by which retinal vascularization is controlled? Be specific, taking into account all that you learned from the previous experiments.

F. (8 points) If you wanted to make an allele of β-catenin to test whether it could suppress a Fz4-/- homozygous null mutant, what genetic properties (i.e., loss of function, gain of function, dominant negative) should it have?

What molecular change could you make to produce such an allele of β-catenin?

Would it matter when and where you expressed this allele? Why or why not?

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Do you predict it would suppress Fz4-/-? Why or why not?