7.06 Cell Biology
EXAM #1
February 28, 2006

This is an OPEN BOOK exam, and you are allowed access to books, a calculator, and notes
BUT NOT computers or any other types of electronic devices.

Please write your answers in PEN (not pencil) to the questions in the space allotted.

Please write only on the FRONT SIDE of each sheet, as we will Xerox all of the exams and thus only grade writing on the front.

And be sure to put your NAME ON EACH PAGE in case they become separated!
There are EIGHT pages including this cover sheet.

Question 1. 23 pts __________
Question 2. 27 pts __________
Question 3. 21 pts __________
Question 4. 9 pts __________
Question 5. 20 pts __________

TOTAL 100 pts __________
Question 1. (23 pts)
You are studying transepithelial transport of glucose from the intestinal lumen to the extracellular medium surrounding the basal surface in cells that are growing in culture. You are using a system identical in set-up to that described for MDCK cells in the textbook, copied below:

(a, 3 pts) If you performed immunofluorescence on permeabilized intestinal epithelial cells using an antibody against GLUT2, where would you find the fluorescent signal?

(b, 5 pts) If you performed the same experiment as above on intestinal epithelial cells that had been depleted of occludin and claudin, where would you see the fluorescent signal? Justify your answer.

(c, 8 pts) You are trying to design a new artificial transporter that would transport glucose from the intestinal lumen into intestinal epithelial cells. You have the ability to create novel transporters and target them to the apical membrane. You make the two new transporters listed below, and to test them you use recombinant DNA techniques learned in 7.02 and 7.03 to express these in the MDCK cells. For each new transporter below, state whether it could allow for movement of glucose across the apical membrane at all, and if yes, whether you think that glucose would move into or out of cells.

(i) A 2 Ca$^{2+}$/1 glucose symporter

(ii) A 2 Ca$^{2+}$/1 glucose antiporter
(d, 7 pts) You expose wild-type mice to a modified version of ouabain. Ouabain inhibits the Na+/K+ ATPase in all body cells, but your version inhibits the Na+/K+ ATPase only in intestinal epithelial cells. You think this might be useful as an antidiabetic drug. Predict the following:

(i) What would happen over time to glucose uptake from the intestinal lumen to the blood and why?

(ii) What would happen over time to the magnitude of the membrane potential of the intestinal epithelial cells?

Question 2. (27 pts)
You are studying the TGF-β pathway in mouse cells growing in culture. Describe how the results obtained from each of the two following experiments would be different, depending on whether or not TGF-β had been added the cells for an hour. Also describe how you would interpret that difference in results (i.e. what the different results in the presence or absence of TGF-β tell you about the effect that TGF-β has).

(a, 7 pts) You perform immunofluorescence on cells using an antibody against Smad-3. (Assume you performed the immunofluorescence correctly, using standard procedures.)

Result without TGF-β:

Result + TGF-β:

Interpretation of difference in results:
(b, 7 pts) You dissolve the cells in a nonionic detergent, remove all remaining membrane fragments by centrifugation, and run the cell lysate over a gel filtration column. You then do Western blotting on each fraction that comes off the column using an antibody against Smad-3.

Result without TGF-β:

Result + TGF-β:

Interpretation of difference in results:

(c, 4 pts) You overexpress in wild-type cells a mutant version of Smad-3 in which its three critical serine residues at the C- terminus – the ones normally phosphorylated by the TGF-β receptor - were replaced with aspartic acid. These cells (in contrast to wild-type cells) rapidly stop growing in culture whether or not TGF-β is added to the cells. Explain this result.

(d, 3 pts) Would the result from part (c) change if the cells had not also been expressing endogenous wild-type Smad-3? If so, how would it change?
(e, 6 pts) Would cells in culture rapidly stop growing if you over-expressed (in wild-type cells) a mutant version of Smad-3 that lacked an NLS…


Question 3. (21 pts)
You form a liposome in buffer #1 that contains 5mM KCl and ATP at pH7. You then place the formed liposome into buffer #2 that contains 50mM KCl and ATP also at pH7. For each subpart below: Describe what would happen very shortly after liposome formation, and then eventually (after a long period of time).

(a, 7 pts) There are no proteins incorporated into the liposome.

(i) Will K\(^+\) move across the membrane, and if so, in which direction does it move?

(ii) What happens to the concentration gradient across the liposome bilayer over time?

(iii) How does the magnitude of the membrane potential across the lipid bilayer change over time?
(b, 7 pts) You now repeat the experiment from the introduction, but using liposomes into which the H\(^+/K^+\) ATPase (isolated from stomach epithelial cells) is incorporated in the same orientation as it is in the cell, with the ATP-binding site inside the liposome.

(i) Will K\(^+\) move across the membrane, and if so, in which direction does it move?

(ii) What happens to the K\(^+\) and H\(^+\) concentration gradients across the liposome bilayer over time?

(iii) How does the magnitude of the membrane potential across the lipid bilayer change over time?

(c, 7 pts) You now repeat the experiment from the introduction, but using liposomes into which only a K\(^+\) channel is incorporated.

(i) Will K\(^+\) move across the membrane, and if so, in which direction does it move?

(ii) What happens to the concentration gradient across the liposome bilayer over time?

(iii) How does the magnitude of the membrane potential across the lipid bilayer change over time?
Question 4. (9 pts)
You are studying muscle contraction. When muscles receive the signals from neurons to contract, calcium is released into the cytosol from an organelle called the sarcoplasmic reticulum (SR), which stores calcium. The sarcoplasmic reticulum contains abundant amounts of a P-class Ca\textsuperscript{2+} ATPase.

(a, 5 pts) What would be the effect over time on muscle contraction if you added a drug that inhibited the Ca\textsuperscript{2+} ATPase in the SR, and why?

(b, 4 pts) There are calcium channels in the membrane of the SR. Do you think that those channels are non-gated or gated? Justify your answer.

Question 5. (20 pts)
Adipocytes (fat cells) store fatty acids as triacylglycerol – a molecule of glycerol (HO-CH\textsubscript{2}–C\textsubscript{3}H\textsubscript{5}OH- CH\textsubscript{2}OH) esterified to three long chain fatty acids. Lipase, the enzyme that hydrolyzes triacylglycerols (TAGs) to one molecule of glycerol and three long chain fatty acids, is found mainly in adipocytes. It is phosphorylated by Protein Kinase A (PK-A) and is catalytically active only when phosphorylated.

You are studying a line of cultured adipocytes that express the G-protein-coupled receptor for adrenaline that is coupled to G\textsubscript{s\alpha}.

For parts (a) – (d) below, state whether the following conditions would result in either:
  constitutively high levels of TAGs,
  constitutively low levels of TAGs,
  high levels of TAGs in the absence of adrenaline but low levels in its presence, OR
  low levels of TAGs in the absence of adrenaline but high levels in its presence.

Explain your choice.
(a, 5 pts) In addition to the wild-type β-adrenergic receptor, your cells express a mutant β-adrenergic receptor (containing three amino acid changes in the cytosol-facing C-terminal segment) that is a GEF for Gsα regardless of the presence or absence of adrenaline.

(b, 5 pts) A mutation in the catalytic domain of PK-A that prevents dissociation from the regulatory domains even if 3’-5’-cAMP is present. (No wild-type PKA is present.)

(c, 5 pts) A mutation in IP (the inhibitory subunit of phosphoprotein phosphatase) that prevents its phosphorylation by PK-A. (No wild-type IP is present.)

(d, 5 pts) You incubate the cells in growth medium to which non-hydrolyzable GTP has been added.