Biology 200
Sample Final Exam
Total time: 2.5 hours.

Section: _____

Family Name: _____ Given Name: _____Student #: _____
(please print)

Instructions:

1. Use black or blue PEN only. Exams that are written in pencil, or that have corrective tape on them will not be eligible for re-grading.
2. Answer **all questions** on this paper. No booklet is required for this exam
3. You are allowed an 8.5x11 inch, double-sided, hand-written memory aid for this exam. All memory aids that do not conform to these rules will be taken away.
4. You will have 75 minutes for this exam.
5. Please return all pages, including rough notes when you hand in your exam.

Codon table for your reference:

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU	UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 7-24 Essential Cell Biology 3/e (© Garland Science 2010)

Question 1. (18 marks)

For each of the observations below, write a statement that could explain these results, based on what the data shows and on your knowledge of cellular structure/ function (3 marks each)

A. If taxol is added to cells at metaphase, the chromosomes do not move towards the spindle poles in anaphase.
Depolymerization of microtubules is the mechanism by which chromosomes move to the spindle poles (during anahase A) (1.5 mark). Taxol inhibits MT depolymerization, which stabilizes MTs thus inhibiting Anaphase A (1.5 mark)

B. Electron micrographs show that mitochondria in heart muscle have a much higher density of cristae than mitochondria in skin cells.
Mitochondria in cardiac cells have more cristae than those of skin cells so that they can house more ATP synthases and produce more ATP (1.5 mark) to meet the higher energy demands of the heart muscle (1.5 mark), in comparison with skin cells.

C. Microtubules formed in vitro from tubulin that is bound to a non-hydrolyzable form of GTP were found to be exceptionally stable.
If GTP cannot be hydrolyzed, then all of the tubulin is in GTP-bound form (1.5 mark). GTP-bound tubulin has higher affinity (binds more tightly) with each other, and thus is more stable in polymer form (1.5 mark).

D. If mRNA for a secretory protein is translated *in vitro* in the absence of any cell membranes, the protein formed was slightly larger than the protein from the same mRNA translated in an intact cell.
Secretory proteins have an N-terminal signal sequence (1.5 mark), which is not cleaved off when the protein in translated *in vitro*, leading to a larger protein *in vitro* compared to the same protein synthesised *in vivo* (1.5 mark)

Alternate wording: Secretory proteins must have an N-terminal signal sequence (1.5 mark). The signal sequence is normally cleaved off in the ER by signal peptidase, but this does not happen in the *in vitro* system.

E. Some membrane proteins can be readily extracted with 1M NaCl, whereas others require the use of an organic solvent or detergent.
Peripheral membrane proteins are held to the membrane via weak, non-covalent/intermolecular interactions, which can be easily disrupted by salts (1.5 mark), whereas integral membrane proteins have a hydrophobic region embedded in the core of the membrane that cannot be removed unless the membrane itself is disrupted using detergents or solvents (1.5 mark).

F. The pH of the chloroplast thylakoid space (or lumen) decreases in light.

Energy from light is used to pump hydrogen ions actively into the thylakoid lumen/space from the stroma via the ETC during photophosphorylation (1.5 mark). The higher hydrogen ion concentration in the thylakoid space in light results in a decrease in pH in this compartment (1.5 mark)

Question 2. (**10 marks**)
When the gene that encodes for Green Fluorescent Protein (GFP) is introduced into cells, GFP is expressed and localized in the cytoplasm of the cell. What specific sorting signals would you have to add to the GFP protein genetically to produce a protein that is targeted to the following final cellular destinations?

	Sorting signal(s) to target the GFP to this location: 2 marks each
A. The nucleus	**Nuclear localization signal (NLS)**
B. The lysosome	**N-terminal signal sequence + mannose-6-phosphate tag**
C. The lumen of the ER	**N-terminal signal sequence + KDEL (retention signal)**
D. The stroma of the chloroplast	**N-terminal chloroplast targeting sequence**

Question 3. (6 marks)
In this course we have used the term polarity in many different contexts; three examples are given below. For each context in the table, explain the meaning of the word 'polar' or 'polarity', and explain how polarity contributes to the function of each example.

Context/example	Definition of 'polarity' in this context (1 mark each)	How this polarity contributes to the function of this example (1 mark each)
A. Polarity in a macromolecular polymer (such as a protein or nucleic acid)	Macromolecules are made up of monomers that have different reactive groups on either end, which is termed polarity (1 mark). **Also possibly acceptable:** directionality of a polymer, the ends are chemically different	This enables polymerization since each time monomers are joined, the reactive ends are maintained to allow further polymerization (1 mark). Allows for clear directionality (e.g. DNA) – information is read in one direction only.
B. Polar amino acid side chains	Polar amino acids are hydrophilic due to charge distribution (1 mark), **OR:** These amino acids have covalent bonds where bonding electrons are attracted more strongly to specific atoms, creating an uneven distribution of electric charge (1 mark).	and spontaneously form H bonds with water and other polar molecules (1 mark). **OR:** This partial charge allows atoms in polar amino acids to readily form H-bonds (1 mark).
C. Polar microtubules	The two ends of a microtubule are structurally distinct due to the orientation of alpha and beta tubulin **OR** having a minus and a plus end (1 mark).	The structural differences influences polymerization dynamics OR gives polymers directionality for motors to move along (1 mark).

Question 4. (10 marks)
You have discovered a new toxin, toxin X, which kills parasitic fungi.

A. You hypothesize that this toxin affects mitosis (M-phase). How would you test this hypothesis? (2 marks)

- There are multiple possibilities, but the simplest method is to use light microscopy to look for cells in mitosis.

B. You find that the cells were not blocked in M-phase. In addition, you discover that this toxic chemical prevents phosphorylation of the fungi's M-cyclin dependent kinase (M-CDK). At what stage of the cell cycle would the fungal cells arrest if toxin X prevents M-CDK phosphorylation? (1 mark)

- The fungi would be arrested in the G2/M checkpoint, also called the CD (commitment of division) checkpoint (1 mark).

Design an experiment to test at what stage of the cell cycle the fungi arrest. State your hypothesis, experimental design and expected results. (7 marks)

- (1 marks) Hypothesis: the cells are arrested in G2.
- Any reasonable experiment to test this hypothesis will be accepted if properly explained. One example is shown below:
  - (4 marks) Experiment: Use FACS to distinguish between G1 from G2
  - (2 marks) Expected results: FACS data would show twice the amount of DNA if cells in G2.

Question 5. (7 marks)
The transferrin receptor is a transmembrane protein involved in the uptake of iron by receptor-mediated endocytosis.

A. Describe the localization pattern you would expect to see in a normal cell by immunoflorescence microscopy if you use a fluorescent antibody against the transferrin receptor.

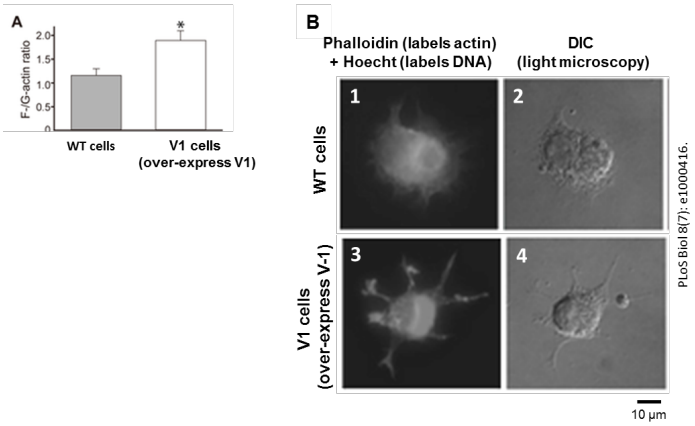
The transferrin receptor would be located at the plasma membrane, vesicles budding from the plasma membrane, endosome, and vesicles budding from the endosome. (**1 mark for each location, total 4 marks**).

B. How would this labeling pattern be changed in a mutant cell in which the transferrin receptor fails to bind to adaptin/clathrin complexes? (2 marks) Why? (1mark)

If the transferrin receptor cannot bind to adaptin/clathrin it cannot be internalized and it would be located only at the plasma membrane (**2 marks**). For receptor mediated endocytosis vesicle formation requires the adaptin/clathrin complex to bind to the cytosolic part of the receptor (**1 mark**).

Question 6 (11 marks)

Actin Capping Protein (ACP) binds to the plus end of actin filaments, preventing the actin filaments from gaining or losing monomers. Its activity is blocked by regulatory proteins such as V-1.
In this experiment, Takeda et al. (2010) examine the role of V-1 and ACP in the regulation of actin polymerization. In Panel A, the ratio of F-actin (actin filaments) to G-actin (actin monomers) were measured for WT (normal) and V1 (over-expresses V-1) cells. In Panel B, actin filaments and nuclei were stained with fluorescent dyes and cells were examined through fluorescence and light microscopy. Assume that both cell lines express the same amount of ACP.



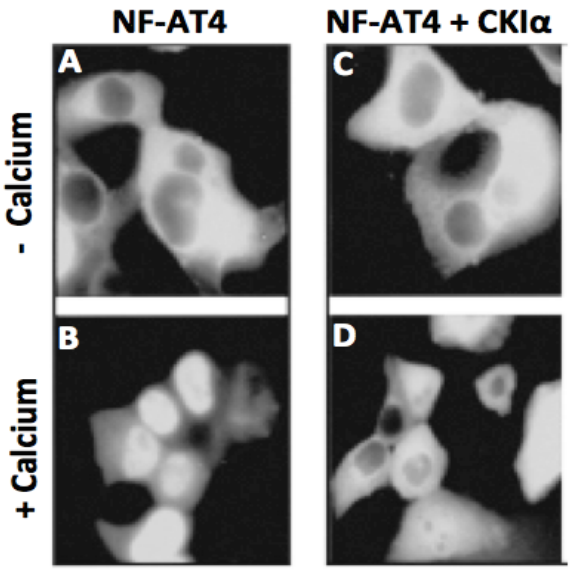
A. Describe the data shown in A (1 mark) and explain what they mean (2 marks).
WT cells have roughly equal amounts of F-actin and G-actin (0.5 marks). V1 cells have roughly twice as much F-actin as G-actin (0.5 marks). **OR** V1 cells have higher ratios of F/G actin than WT cells—they have more actin filaments than WT cells. (1 mark for the comparison)
ACP prevents actin from gaining or losing monomers. V1 inhibits ACP allowing actin to polymerize (1 mark) resulting in more F-actin than G-actin (1 mark)

B. Describe the data for each panel shown in B (2 marks) and explain what they mean (2 marks).
Panel B, images on the top panel show the control. Left Image shows the wild type distribution of actin in the cell. Image on the right shows the wild-type shape of the cell. Actin is distributed evenly throughout a spherical cell.
In the lower panels, Image on the left shows the distribution of actin throughout a cell overexpressing V1, and image to the right shows the shape of the cell. The cell has changed shape, and now has several protruding filopodia/lamellipodia.
1 mark each for describing the actin distribution and the shape and 1 mark each fro describing what it means.

C. Based on the data shown in A and B, what accounts for the changes in cell shape that are seen between the WT and V1-over-expressing cells? (2 marks)
In V1 cells, actin polymerization is enhanced/increased (1 mark) resulting in more filaments pushing the plasma membrane out in several directions to create multiple filopodia?/lamellipodia.(1 mark)

D. What can you conclude about the role V-1 has in actin polymerization? (2 marks)
V1 increases actin polymerization by inhibiting ACP and allowing G-actin to polymerize to F-actin

Question 7 (10 marks)
NF-AT4 is a transcription factor that is regulated by calcium. Here, Zhu et al. (1998) use GFP-tagged NF-AT4 to establish that NF-AT4 function is also regulated by CKIα kinase, a protein that adds phosphate groups directly to NF-AT4.



Adapted from Cell 93:851

A. Consider panels A and B. Describe what the data show and explain the effect that the addition of calcium has on the NF-AT4 protein. (3 marks)

Panel A. In the absence of calcium, NF-AT4 is visible/localized in the cytosol of the cell. (1 mark)

Panel B. When Ca+ is added, most of NF-AT4 becomes visible in the nucleus (1 mark)

This means that the addition of Ca+ has caused NF-AT4 to relocate to the nucleus. (1 mark)

B. Consider panels C and D. Describe what the data show and explain how the presence of CKIα impacts the effect of calcium on calcium-dependent NF-AT4. (3 marks)

Panel C - When CKIa is added in the absence of Ca+, NF-AT4 is visible in the cytosol OR Panel C similar to Panel A (1 mark).

Panel D - When CKIa is added in the presence of Ca+, NF-AT remains visible in the cytosol instead of becoming visible in the nucleus OR there is reduced translocation of NF-AT4 to the nucleus OR Panel D is similar to Panel A OR is dissimilar to Panel B (1 mark).

suggests that CKIa suppresses Ca-induced NF-AT4 translocation to the nucleus (masks the NLS that would otherwise be exposed by Ca signalling OR CK1a blocks the effects of Ca) (1 mark)

C. Based on the data above propose a model for how calcium and CKIα can influence the localization of NF-AT4 in these cells? (2 marks)
Possibilities include:
- Ca+ causes an NLS to be exposed on NF-AT4 (or causes the NLS to be recognized or allows protein to fold in final/native conformation), allowing for translocation to the nucleus.
- CKIa phosphorylation blocks recognition of the NLS and thus blocks translocation to the nucleus, even when calcium is present.
- Ca blocks nuclear export or NES receptor

D. Why would addition of a phosphate group to NF-AT4 alter its function? (2 marks)

Addition of the phosphate group could alter how NF-AT folds, altering the shape and function of the NLS/masking the presence of the NLS OR induces change in conformation/shape that affects function.

Question 8 (essay outline. 20 marks)
Each organelle in the cell is a unique microenvironment where certain cellular functions are performed. Write an essay outline to critically assess the following statement: "The intracellular areas of low pH are essential to the function of some organelles." Your arguments (and associated supporting evidence) should provide examples from 3 different organelles.

Marking scheme:

Organization - 6 marks

- 2 marks – Spelling/grammar
- 1 mark – length of argument/ evidence (should only be ~3 sentences in each box)
- 1 mark – outline format (i.e. bullet points and not paragraphs)
- 2 marks – Clarity and flow

Thesis statement (2 marks)
A good thesis statement should include:

- A statement about how essential areas of low pH are to organelle function
- A statement that identifies what's going to be in each of the 3 boxes that follow (either a list of organelles or a list of functions. Both are not required)

Argument and supporting evidence (4 marks each)
They need to talk about 3 different organelles for this question (1 per box).
4 marks per argument/ evidence include:
1 mark – Argument sentence (i.e. a sentence to summarize what they're trying to argue in that box).
1 mark – Identify where in the organelle/ structure the are of low pH is.
2 marks – An explanation of how the low pH is used to perform a function

- Includes 1 mark for connecting function to low pH, and 1 mark for additional detail about the function.

Some examples of different organelles and how they use pH are below, grouped together by Unit.
Note: terms that are acceptable to describe pH include acidic/ basic, high/ low pH, high/low proton concentrations (i.e. proton gradient), high/ low Hydrogen (H^+) ion concentration.

Mitochondria:

- Area of low pH: intermembrane space
- Primary function related to low pH: ATP synthesis
- Additional details (not an exhaustive list. Student needs 2 and they should be integrated together):
    - ETC pumps protons into intermembrane space from matrix
    - Protons flow through ATP synthase to drive ATP synthesis
    - Proton gradient established across membrane (i.e. areas of high/ low pH)
    - If gradient is lost, no ATP is formed
    - If gradient is reversed, ATP will be hydrolysed to and protons will be pumped into intermembrane space.

Chloroplast:

- Area of low pH: Thylakoid lumen
- Primary function related to low pH: ATP synthesis
- Additional details (not an exhaustive list. Student needs 2 and they should be integrated together):
    - ETC pumps protons into thylakoid lumen/ space
    - Protons flow through ATP synthase to drive ATP synthesis
    - Proton gradient established across membrane (i.e. areas of high/ low pH)
    - If gradient is lost, no ATP is formed
    - ATP produced in the stroma is used to make carbohydrates.

Endomembrane system
Golgi:

- Area of low pH: Trans Golgi Network
- Primary function related to low pH: protein aggregation for regulated secretion
- Additional details (not an exhaustive list. Student needs 2):
    - pH gets more acidic as you move from cis to trans
    - Protein aggregation helps with vesicle formation
    - Proteins destined for constitutive secretion do not aggregate at low pH
    - Students may talk about pH and protein processing in the Golgi. Changes in pH can cause conformation changes, but this is not the same as protein processing, so no marks should be awarded for this idea.

Both endosomes and lysosomes have a low pH. If students choose to discuss both of these organelles, there may be some overlap in the ideas use to support each argument. This is fine, as long as the focus of the primary focus of the information is related to the organelle being discussed.

Endosome:

- Primary function related to low pH: receptor/ cargo dissociation
- Additional details (not an exhaustive list. Student needs 2):
    - Receptor-mediated endocytosis is used to bring in receptors and cargo
    - Lysosomal enzymes are sent to the (late) endosome on their way to the lysosome
    - Separation of cargo from receptor in (early) endosome allows receptor to be recycled (this could be either to the PM or back to the TGN, depending on which receptors they chose)
    - Endocytic cargo will eventually end up in lysosome, where it will be digested.
    - Lysosomal enzymes will eventually end up in lysosome, where they will get activated and start to digest things.
    - Proton pumps used to acidify endosomal compartment.

Lysosome:

- Primary function related to low pH: enzyme activation
- Additional details (not an exhaustive list. Student needs 2):
    - Digestive enzymes need to be in low pH in order to be functional/ activated.
    - Proton pumps used to acidify compartment
    - Low pH activation of digestive enzymes can act as a way to protect the cell in case of mistargeting of the lysosomal enzymes (e.g. cytosol is neutral, so enzymes cannot work there)
    - pH gets increasingly acidic as protein moves through the endomembrane (ER to Golgi to endosome to lysosome.
    - Material that is to be digested most often is brought in through the endosome, via endocytosis.

Vesicles: This is also an area of low pH in the cell.

There may be other areas of low pH that are identified, and must be assessed on a case-by-case basis.

Areas that do NOT have a low pH, and thus cannot be used in this essay outline:

- Cytosol (pH 7.3)
- Nucleus (also pH 7.3).