CELL BIOLOGY, BLG311 **INSTRUCTOR: DR. ROBERTO BOTELHO**

STUDENT NAME: **STUDENT NUMBER:**

Mid-term test 1: VERSION 1

Part A: Answer all 15 multiple-choice questions all worth 1 point = total 15

Part B: Answer 5 simple questions all worth 2 points = 10. Answer a sixth for 2 bonus points.

Part C: Answer only 3 written questions all worth 6 points = total 18

Make sure to follow the instructions:

1. Enter your name and student number ABOVE
2. Enter you name and student number in the scanning sheet (write and fill the bubbles)
3. **MAKE SURE TO WRITE YOUR TEST VERSION ON THE TOP OF YOUR SCAN SHEET!**
4. **Failure to follow instructions may result in up to a 10% deduction.**

PART A: Multiple-choice

1. **Transcription refers to the process of:**
   a. Replicating DNA
   b. Synthesizing protein using mRNA as the code
   c. Synthesizing RNA using DNA as the code
   d. Synthesizing macromolecules
   e. Synthesizing nucleic acids from the protein code
2. **Nucleotides are polymerized into nucleic acids. The bond that joins individual nucleotides together is called:**
   a. The peptide bond
   b. The phosphoester bond
   c. The glycosidic bond
   d. The peptone bond
   e. The carboxylic ester bond
3. **What is deconvolution?**
   a. A technique that uses light phase-contrast
   b. A software algorithm that polarizes light
   c. A software algorithm that de-blurs fluorescence images of cells
   d. A technique to calculate recovery rates of fluorescence
   e. None of the above

4. **As a Ph.D. student you engineered a protein fusion between actin and a photo-activatable GFP. What kind of experiment could you do with this fusion that you could NOT do with an actin fusion to the "standard GFP"?**
    a. Localization of actin
    b. Diffusion rates of actin
    c. Gene expression of actin
    d. Actin interaction with GFP
    e. None

5. **The modern eukaryotic cell likely evolved in three distinct and successive steps. Which of the following is the likely order of these events?**
    a. Membrane involution, followed by chloroplast evolution, followed by mitochondria evolution
    b. Membrane involution, followed by concurrent chloroplast and mitochondria evolution
    c. Membrane involution, followed by mitochondria evolution, followed by chloroplast evolution
    d. Chloroplast evolution, was followed by mitochondria evolution, followed by membrane involution
    e. Membrane involution, chloroplast evolution, and mitochondria evolution all occurred concurrently

6. **Which condition below would produce the worst resolution:**
    a. 400 nm light and a numerical aperture of 1.5
    b. 450 nm light and a numerical aperture of 1.2
    c. 400 nm light and a numerical aperture of 1.3
    d. 450 nm light and a numerical aperture of 1.3
    e. 500 nm light and a numerical aperture of 1.2

7. **What is TRUE about confocal microscope?**
    a. Confocal microscopy serves to remove out-of-focus light
    b. Confocal microscopy uses a laser to scan the sample and generate an image
    c. Confocal microscopy can acquire multiple images along the z-axis to create a 3D model of the sample
    d. Confocal microscopy uses pinholes to block out-of-focus light from the detector
    e. All are true

8. **As a Ph.D. student you engineered a protein fusion between tubulin and GFP. What kind of information can you obtain with this fusion protein?**
    a. Localization of tubulin in living cells
    b. Diffusion rates of tubulin molecules
    c. Localization of tubulin-GFP within 20 nm resolution
    d. Measure the concentration of tubulin in cells
    e. All of the above

9. **Which statement about the green fluorescent protein (GFP) is TRUE:**
    a. The gene for GFP was isolated from a coral
    b. There are alleles/mutants of GFP that emit other colours
    c. GFP can be used to visualize proteins by electron microscopy
    d. GFP is excited by green light
    e. GFP fusion to proteins of interest occurs non-covalently

10. **Which methods could you employ to simultaneously deliver plasmid DNA into thousands of cells?**
    a. Microinjection or Electroporation

b. Microinjection or Carrier-Vesicle Fusion
c. Electroporation or Carrier-Vesicle Fusion
d. Any of the above
e. None of the above

11. Which of the following electron microscopy techniques allows direct observation of organic samples?

a. Scanning electron microscopy
b. Cryo-electron microscopy
c. Immunogold electron microscopy
d. Deep-etching and metal shadowing
e. All of the above

12. GTPase-activating proteins (GAPs) are:

a. Proteins that stimulate the phosphorylation of GDP back to GTP to activate GTPases
b. Proteins that displace GTP and permit GDP to bind to GTPases
c. Proteins that stimulate the exchange of ADP for ATP binding by GTPases
d. Proteins that stimulate GTP hydrolysis by GTPases
e. Proteins that displace GDP and permit GTP to bind to GTPases

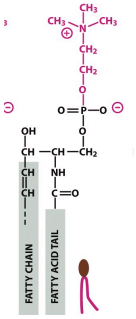
13. Which statement if FALSE about membranes:

a. Membrane bilayers of different organelles can have different compositions
b. Membrane bilayers are made of cylindrical-shaped phospholipids
c. The two membrane leaflets of a bilayer can be different in composition
d. Lipids are uniformly distributed in membrane bilayers
e. If there are no false statements above, then choose "e".

14. Which phospholipid has a negative net charge?

a. Phosphatidylserine
b. Phosphatidylcholine
c. Phosphatidylinositol
d. Sphingomyelin
e. Phosphatidylethanolamine

15. What type of phospholipid is shown?



a. glycerophospholipid
b. phosphatidylserine
c. sphingophospholipid
d. conical phospholipid
e. cholesterol

Part B: SIMPLE questions. Answer 5 out of eight questions. Answer a sixth question for up to 2 BONUS points. 2 points per question = 10 points + 2 bonus.

1. **Define "peptide bond".**

A peptide bond covalently joins the carboxyl terminus of one amino acid to the amino-terminus of a second amino acid /1.5.
Essential to form polypeptides (or make proteins or makes polymers of amino acids) /0.25
This occurs by a condensation reaction /0.25

2. **Briefly describe the symbiotic relationship between a primordial "eukaryotic cell" and the bacteria that gave origin to "mitochondria". Make sure to identity the type of bacteria.**

Primordial eukaryotic cells developed a symbiotic relationship with oxidizing bacteria (/0.5)
Symbiosis was such that the eukaryotic cell provide food molecules (likely by hunting, phagocytosis) /0.75
while the bacteria provided the ability to oxidize food molecules to make ATP /0.75.
Over time, oxidizing bacteria evoled into mitochondria.

3. **What is the primary structure of a protein? State what the primary structure of a protein determines.**

Primary structure of a protein is the order/sequence of amino acids that make up the protein /1
Primary structure determines the folding of the protein, /0.5
which determines the function of the protein /0.5

4. **What is an endergonic reaction? State how cells drive endergonic reactions forward.**

An endergonic reaction is a reaction that has a positive ΔG /1
This means that the reaction requires energy 0.25 and is not spontaneous /0.25
Cells drive these reactions forward by coupling to exergonic reactions. /0.5

5. **What is fluorescence recovery after photobleaching?**

Fluorescence recovery after photobleaching (FRAP) is a technique whereby a laser is used to photobleach (destroy) the fluorescence signal. /1
Photobleaching is usually done for a small region of the cell leaving the rest of the fluorescence intact. /0.25
This is useful to study molecular diffusion /0.75

6. **Define fluorescence (I am NOT asking about the set up of a fluorescence microscope).**

Fluorescence is a process by which a photon of a specific wavelength/energy is able to excite/be absorbed by certain compounds/fluorochromes. /1
These compounds then release/emit a photon of specific longer wavelength/lower energy. /1

7. **Briefly describe the difference between magnification and resolution.**

Magnification is a process by which an object is made to appear larger. /1
Resolution is a process by which one can distinguish more detail/information about an object that is made to appear larger. /1

8. **Briefly state the commonly and infrequently observed movements of phospholipid molecules in a lipid bilayer.**

Phospholipids in a bilayer can undergo lateral diffusion (0.5), flexion (fatty tails bend /0.5), or rotation (0.5)
These are all frequent movements.

Infrequent movement is flip-flopping, where the phospholipid moves from one leaflet to the other /0.5

PART C: Mid-complexity questions. Answer 3 out of the 4 questions. 6 points each = 18 points total.

1. **Define protein phosphorylation and dephosphorylation. A) Make sure to identify the key enzymes, substrates and products and; B) Make sure to state the general function of protein phosphorylation and dephosphorylation – i.e., what is the effect on the substrate protein.**

Protein phosphorylation refers to the covalent modification of a protein by the addition of a phosphate. /0.75
Dephosphorylation refers to the removal of the phosphate from the protein /0.75

A. Protein phosphorylation is catalysed by protein kinases. The substrates of this reaction are protein and ATP and the products are ADP and phospho-protein /1.25
Dephosphorylation is catalysed by protein phosphatases. The substrates are phospho-proteins plus water and products are dephosphorylated protein and phosphate. /1.25

B. Protein phosphorylation and dephosphorylation is used to change the structure of a protein so that it is either active or inactive. /1

Diagrams are acceptable to help explain

2. **Discuss indirect immunofluorescence by: a) Explaining what it is used for; b) Explaining how it works (don't bother writing about B-cells and how cells make antibodies in your answer – simply how indirect Immunofluorescence works) and; c) Make sure to state what processing must be done to cells for immunofluorescence to work.**

a. II is used to localize specific proteins in a cell. /1

b. II works by:

- incubating cells with a primary antibody, which binds to the specific protein of interest (antigen). /1
- This is followed by a secondary antibody, which binds to the primary antibody. /1
- The secondary antibody is modified with a signal emitting molecule like a fluorochrome /1
- This way signal is emitted where the antigen is present in the cell. /0.5
- Secondary antibodies are used because they help amplify the signal 0.5

c. Before staining with primary and secondary antibodies, cells must be fixed (/0.5) with a chemical to cross-link proteins in place and then permeabilized /0.5

3. **With respect to transmission electron microscopy: A) describe how cells are processed for transmission electron microscopy; B) explain how transmission EM works and C) what is the key reason why one uses electron microscopy over light microscopy?**

   A. Cells are processed by:
   - Cells are first fixed to cross-link molecules in place /1
   - Samples are then sectioned with a microtome into very thin slices (50-100 nm) /0.5
   - Cells are then treated with heavy atom metals to give contrast by creating a metal cast/copy /1
   - Sometimes cells are flash-frozen /0.5

   B. TEM works by shooting an electron beam through the processed cells. The electrons are either reflected or pass through depending on how the heavy metals coated the sample. The differential passage/transmission of electrons through the sample creates an image of the cell /2

   C. One would use TEM instead of light to obtain very high resolution images of the sample (less than 200 nm) /1

4. **With respect to membrane structure: A) Identify the major lipid constituents of the membrane bilayer; B) Draw the general structure of a glycerophospholipid (identify the major bonds and moieties) and C) Explain the effect on membrane fluidity and permeability by increasing the cholesterol concentration of the bilayer?**

   A. Major lipid constituents of the membrane are phospholipids (0.5), which can be composed of glycerophospholipids (0.5) and sphingolipids (0.5), and of sterols/cholesterol (0.5).

   C. Cholesterol is a planar, hydrophobic molecule. It can intercalate between phospholipids fatty acid chains to increase the density of the membrane/reduce "empty" space. Thus, this decreases fluidity (more packed) and decreases permeability to water. /2

   B.

True… it's the end… I'm quite sorry… don't cry.

3. **With respect to transmission electron microscopy: A) describe how cells are processed for transmission electron microscopy; B) explain how transmission EM works and C) what is the key reason why one uses electron microscopy over light microscopy?**

   A. Cells are processed by:
   - Cells are first fixed to cross-link molecules in place /1
   - Samples are then sectioned with a microtome into very thin slices (50-100 nm) /0.5
   - Cells are then treated with heavy atom metals to give contrast by creating a metal cast/copy /1
   - Sometimes cells are flash-frozen /0.5

   B. TEM works by shooting an electron beam through the processed cells. The electrons are either reflected or pass through depending on how the heavy metals coated the sample. The differential passage/transmission of electrons through the sample creates an image of the cell /2

   C. One would use TEM instead of light to obtain very high resolution images of the sample (less than 200 nm) /1

4. **With respect to membrane structure: A) Identify the major lipid constituents of the membrane bilayer; B) Draw the general structure of a glycerophospholipid (identify the major bonds and moieties) and C) Explain the effect on membrane fluidity and permeability by increasing the cholesterol concentration of the bilayer?**

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   C. Cholesterol is a planar, hydrophobic molecule. It can intercalate between phospholipids fatty acid chains to increase the density of the membrane/reduce "empty" space. Thus, this decreases fluidity (more packed) and decreases permeability to water. /2

   B.