BSc Degree Examinations 2019-0

Department: Biology

Title of Exam: Cell Biology

Time Allowed: 24 hours
(Please note: Late papers will not be marked)

Time Recommended: 2 hours and 30 minutes

Word limit: Please answer the question in the line limit stated. Content beyond the line limit will not be marked.

Allocation of Marks:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper.

Instructions for Candidates:
Please answer all questions within the stated line limit.
Please use Arial font, size 11 or larger. Do not adjust the margin width.
A note on Academic Integrity

We are treating this online examination as a time-limited open assessment, and you are therefore permitted to refer to written and online materials to aid you in your answers.

However, you must ensure that the work you submit is entirely your own, and for the whole time the assessment is live you must not:

- communicate with departmental staff on the topic of the assessment
- communicate with other students on the topic of this assessment.
- seek assistance with the assignment from the academic and/or disability support services, such as the Writing and Language Skills Centre, Maths Skills Centre and/or Disability Services. (The only exception to this will be for those students who have been recommended an exam support worker in a Student Support Plan. If this applies to you, you are advised to contact Disability Services as soon as possible to discuss the necessary arrangements.)
- seek advice or contribution from any third party, including proofreaders, friends, or family members.

We expect, and trust, that all our students will seek to maintain the integrity of the assessment, and of their award, through ensuring that these instructions are strictly followed. Failure to adhere to these requirements will be considered a breach of the Academic Misconduct regulations, where the offences of plagiarism, breach/cheating, collusion and commissioning are relevant - see AM.1.2.1” (Note this supersedes section 7.3 of the Guide to Assessment).
1. Why do eukaryotic cells have different cytoskeletal elements (microfilaments, microtubules and intermediate filaments) rather than just one type? (6 lines maximum, 3 marks)

2. Which of the following cells would you expect to contain a high density of intermediate filaments (select all that apply)? Explain your answer. (8 lines maximum, 5 marks)

   A. Amoeba proteus (a free-living amoeba)
   B. Human skin epithelial cell
   C. Smooth muscle cell from the digestive tract of a vertebrate
   D. Nerve cell from a mouse spinal cord
   E. Human sperm cell

3. The following graph shows growth rates at the plus and minus ends of actin filaments as a function of actin concentration.
a) Complete the table below to indicate whether the plus and minus ends of an actin filament would grow, shrink or remain unchanged if added to a solution of G-actin of each of the concentrations indicated as A, B, C, D and E.  

<table>
<thead>
<tr>
<th>(+) end</th>
<th>(-) end</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

b) Would treadmilling occur at any of these concentrations? Explain your answer.  

4. Explain why it could be detrimental for an organism to have mutations in connexins that render GAP junctions unable to a) open or b) close.  

5. Compare and contrast the similarities and differences in activation and inactivation for the signal transduction steps: (i) using an SH2 domain, (ii) leading to the activation of PKA, (iii) leading to the activation of a trimeric G-protein.  

6. While investigating a new type of muscle cell, you hypothesize that signal transduction from alpha adrenergic receptors on the cell surface to calmodulin activated kinase (CAM kinase) is responsible for the increased mitochondrial activity that supports muscle contraction. Outline an experimental strategy to test this hypothesis using an oxygen electrode that can measure activity of isolated mitochondria. Your answer should include appropriate controls and an explanation of why you have chosen the different experimental steps in your strategy.
7. While investigating the receptor CB011 in HeLa cells, you observe that it localizes to the plasma membrane. The receptor is tagged with GFP, and you use this to perform experiments of photobleaching followed by recovery of the fluorescence in the bleached area. Results are in the figure below.

![Fluorescence recovery after photobleaching](image.png)

**Figure 1.** Fluorescence recovery after photobleaching of CB011-GFP in HeLa cells. Photobleaching was at 0 seconds, as marked by the arrow. Cells were treated to remove cholesterol or glycolipids as indicated.

a) Based on the results of Figure 1 and your knowledge of the fluid mosaic model of membranes, what can you conclude about the localization of CB011? Explain your answer.  
(10 lines maximum, 5 marks)

b) Based on Figure 1, approximately what percentage of the plasma membrane was bleached in the experiment? Explain your answer.  
(2 lines maximum, 2 marks)

c) Glycolipids face the outside of the cell, although the first steps of glycolipid biosynthesis are on the cytosolic face of the ER. Briefly explain how a glycolipid can move from its initial site of synthesis to its final destination.  
(5 lines maximum, 3 marks)
8. Cell Type-A, expresses the SNARE proteins Syntaxin-7 (Qa), Vti1b (Qb), and Sec22b (Qc). Cell Type-B, expresses the SNARE protein Vamp 7 (R). Cell Type-C expresses the SNARE protein Vamp 8 (R). In all cases the SNARE proteins were modified so that they become Type-I membrane proteins. Different combinations of cells were incubated together and cell-to-cell fusion was observed as in the table.

<table>
<thead>
<tr>
<th>Cell Types Incubated together</th>
<th>Cell-to-cell fusion observed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A and B</td>
<td>No</td>
</tr>
<tr>
<td>A and C</td>
<td>Yes</td>
</tr>
<tr>
<td>B and C</td>
<td>No</td>
</tr>
</tbody>
</table>

a) Which SNAREs facilitate cell-to-cell fusion? (1 line maximum, 2 marks)

b) Where are the SNAREs which facilitate cell-to-cell fusion localised? (1 line maximum, 1 mark)

c) Would AxC cell-to-cell fusion proceed in the presence of N-ethylmaleimide? Explain your answer. (4 lines maximum, 2 marks)

9. You wish to investigate the properties of a newly identified protein. An in vitro transcription-translation reaction is set up in the presence or absence of microsomes (intact fragments of ER) and -/+ GTP. Following protein production the protein is analysed by SDS-PAGE, with or without the reducing agent DTT. A representation of the experimental outcome is shown below.
a) From these data, what can be concluded about the likely intracellular location of the protein? Explain your answer. (4 lines maximum, 2 marks)

b) Why is the protein smaller in the presence of microsomes and GTP? Explain your answer. (4 lines maximum, 2 marks)

c) What post-translational modifications occur to the protein, as revealed by the DTT treatment? (1 line maximum, 1 mark)

10. Cells from a lysosomal storage disorder patient are found to secrete their lysosomal hydrolases rather than retain them intracellularly in the lysosome. Give 3 potential reasons for why the hydrolases may be secreted. (2 lines each maximum, 3 marks)

   i)  

   ii)  

   iii)  

11. RNA interference (RNAi) was used to deplete cells of either the µ2 subunit of AP-2, or the heavy chain of clathrin. In both cases AP-2 µ2 or the clathrin heavy chain were undetectable. Transferrin or EGF were radiolabelled and endocytosis of these ligands, in control and RNAi cells, was measured by measuring the amount of radioactivity (CPM) inside cells over time. Data are shown by the figure below (key is the same for both graphs).
a) Explain the differences in the uptake of the two ligands in control cells.  
(8 lines max, 2 marks)

b) What molecules are required for endocytosis of the transferrin-receptor?  
(4 lines max, 2 marks)

c) The EGF-receptor is known to bind AP-2 and clathrin for internalisation. Give three potential reasons to explain the data, in graph b, for EGF uptake in AP-2 depleted cells.  
(6 lines max, 3 marks)

12. What are the consequences to the cell of impaired mitophagy?  
(6 lines max, 5 marks)

13. The proapoptotic member of the Bcl-2 family, Bad was found to be phosphorylated on serine residues 112 and 136. This phosphorylation was investigated, by western blotting, in two cancer cell lines (Caov-3 and A2780) after various treatments (results shown below). Cisplatin (but not its isomer Transplatin) induced phosphorylation on both serines. Cells were treated with PI3-kinase inhibitor wortmannin or with ERK kinase inhibitor PD98059 before cisplatin treatment. The western blot shows levels of phospho-Bad112 (A), phosphoBAD136 (B) or total cellular BAD levels (C).
a) Which serine residue is phosphorylated by ERK?  
(1 line maximum, 1 mark)

b) Which serine residue is phosphorylated by Akt?  
(1 line maximum, 1 mark)

c) Bad-Phosphoserine136 is required for Bad to interact with 14-3-3 in the cytoplasm. Cisplatin is a chemotherapy drug. Which compound wortmannin or PD98059 is likely to make cells more sensitive to apoptosis? Explain your answer.  
(6 lines maximum, 3 marks)
14. The schematic below explains an *in vitro* scratch wound assay to analyse cell migration.

**The Scratch Wound Assay**

A scratch wound assay experiment was conducted to determine how keratinocyte migration was affected when expression of Focal Adhesion Kinase (FAK) was knocked down using siRNA.

The table below shows the width of the scratch wound in μm measured in six replicates at time = 0, 24 and 48 hours (h) in Control keratinocytes and FAK knockdown (kd) keratinocytes.

<table>
<thead>
<tr>
<th>Control (0h)</th>
<th>FAK kd (0h)</th>
<th>Control (24h)</th>
<th>FAK kd (24h)</th>
<th>Control (48h)</th>
<th>FAK kd (48h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>410</td>
<td>420</td>
<td>320</td>
<td>350</td>
<td>205</td>
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<tr>
<td>430</td>
<td>415</td>
<td>280</td>
<td>360</td>
<td>190</td>
<td>295</td>
</tr>
</tbody>
</table>
a) Calculate the percentage scratch wound closure for Control and FAK kd keratinocytes at 24h and 48h. (20 lines maximum, 6 marks)

b) What are the effects of FAK knockdown on keratinocyte migration? (1 line maximum, 1 mark)

c) Under what circumstances would a FAK inhibitor be a good anti-cancer drug? (1 line maximum, 1 mark)

d) Assuming that the FAK inhibitor functions by disrupting focal adhesion, would you expect the effect of a FAK inhibitor on cell migration to be enhanced, reduced or unaffected by simultaneous treatment with:

i) an agent that destabilises actin microfilaments? (1 line maximum, 1 mark)

ii) an agent that destabilises microtubules? (1 line maximum, 1 mark)

iii) Explain your answers to i) and ii). (8 lines maximum, 5 marks)

15. Briefly explain how the pluripotent function of embryonic stem cells is controlled at the transcriptional level. (5 lines maximum, 3 marks)

16. An experimental protocol is being designed to differentiate adult limbal stem cells into corneal tissue to help treat impaired eyesight.

a) Provide one advantage and one disadvantage of using adult limbal stem cells compared to induced pluripotent stem cells (iPSCs). (4 lines maximum, 2 marks)

b) Part of the protocol involves growing the limbal stem cells on type IV collagen. Explain why this ECM substrate in particular was chosen. (3 lines maximum, 2 marks)
c) What *in vitro* tests would you use to determine if the limbal stem cells had differentiated into corneal cells? (6 lines maximum, 3 marks)

End of Exam