BSc / MSc Degree Examinations 2018-9

Department:
Biology

Title of Exam:
Cell Biology

Time Allowed:
2.5 hours

Instructions for Candidates:
Answer all questions

Do not write on this booklet before the exam begins
Do not turn over this page until instructed to do so by an invigilator
1. You have developed an assay to follow formation of actin filaments from purified actin monomers. Your assay starts with purified actin monomers labeled with a fluorescent probe. Upon polymerization the fluorescence of the probe increases, allowing polymerization to be measured. Data from a typical time course is shown are plotted on the left hand graph. The right hand graph shows the equilibrium distribution of actin in free subunits (monomers) and in filaments, as a function of actin concentration.

a) Speculate as to why the intensity of fluorescence at time zero is not zero. *(1 mark)*

b) What stages of actin filament assembly do the three phases of the polymerization curve on the left hand graph reflect? *(3 marks)*
   
   (i)
   
   (ii)
   
   (iii)

(c) Estimate the critical concentration of actin polymerization for this experiment. *(1 mark)*

(d) How would the curve on the left change if you doubled the initial concentration of actin monomers used in the assay? Would the concentration of free (monomeric) actin at equilibrium be higher or lower than in the original experiment? *(4 marks)*
2.  

a) Give one example of a physiological process mediated through gap junctions  

(1 mark)

b) What is the main advantage of communication occurring through gap junctions rather than a less direct mechanism?  

(1 mark)

c) Under what circumstances are gap junctions closed and why?  

(2 marks)

d) How is this closure regulated?  

(2 marks)

3.  
At 1.4mg/ml pure tubulin, microtubules formed from 13 protofilaments grow at a rate of 2mm/min. At this growth rate how many ab-tubulin dimers (8nm in length) are added to the ends of a microtubule each second?  

(4 marks)

4.  
Which second messengers are generated downstream of phospholipase C and how?  

(3 marks)
5. How could you use cholera toxin to investigate adrenergic signalling in the heart? (4 marks)

6. Noradrenaline is an adrenaline analogue secreted by the sympathetic nervous system. While it also acts through adrenergic receptors, it can trigger different signalling pathways. In the experiment below glycogen synthesis was monitored in cells treated with noradrenaline and insulin, either with or without the PI3-kinase inhibitor wortmannin.

![Graph showing glycogen synthesis](image)

a) Do insulin and wortmannin act through the same signalling pathway to promote glycogen synthesis? Explain your answer. (4 marks)

b) Propose an experiment that would probe if noradrenaline acts through the inhibitory G-alpha subunit to hinder cAMP signalling in this instance? (4 marks)
7. The following statements are true for either the α or the β adrenergic receptors or both. For each statement indicate whether the answer is α or β or both. (3 marks)

- Affected by first generation beta blockers
- Couples to trimeric G-proteins
- Signals through phospholipase C

8. a) What is the main site of phospholipid biosynthesis? (1 mark)

b) Explain how asymmetry between the two leaflets of the phospholipid bilayer is established? (3 marks)

9. Give two examples of modification that can influence the binding of peripheral membrane proteins to membranes. (4 marks)

10. A patient arrives in the clinic and examination of the patient’s blood identifies high levels of the lysosomal hydrolase cathepsin-B.

   a) Suggest two possible explanations for this observation. (2 marks)
b) The cathepsin-B protein from the patient and a 'normal' individual were subjected to SDS-PAGE and transferred to two membranes. One membrane was western blotted for cathepsin-B (image on the left) and the other membrane was incubated with a mannose-binding lectin and the lectin visualised (image on the right). Explain the observations and how this causes elevated cathepsin-B in the patient’s blood. 

(4 marks)

<table>
<thead>
<tr>
<th>kDa</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>119</td>
<td></td>
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</tbody>
</table>

--western blot--

<table>
<thead>
<tr>
<th>kDa</th>
<th>Control</th>
<th>Patient</th>
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<tbody>
<tr>
<td>120</td>
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<td></td>
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<tr>
<td>119</td>
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</tbody>
</table>

--lectin binding--

c) When cells from the patient were treated with rapamycin, a drug which can induce autophagy, no effects were seen on the availability of nutrients. Given also the data above, suggest what might be wrong with the patient. 

(3 marks)

11. You incubate adipose cells with insulin, which promotes exocytosis of the glucose transporter GLUT4. The extracellular concentration of glucose, before insulin addition, is 10 mM. The cells after insulin addition, remove 50% of the extracellular glucose per minute.

a) Plot a graph of extracellular glucose concentration vs time for the 5 minutes after the addition of insulin. 

(4 marks)
The binding of insulin to its receptor causes the receptor to be endocytosed and degraded by the lysosome. To increase insulin sensitivity and subsequently glucose uptake, cells were treated with various compounds with the aim to inhibit insulin-receptor endocytosis. Glucose uptake was measured as above.

The following table shows the drugs used and the rate of glucose uptake.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_{\frac{1}{2}}$ glucose uptake (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynasore (inhibits dynamin)</td>
<td>0.5</td>
</tr>
<tr>
<td>Nocodazole</td>
<td>1</td>
</tr>
<tr>
<td>Vanadate (phosphatase inhibitor)</td>
<td>2</td>
</tr>
<tr>
<td>SP600125</td>
<td>1</td>
</tr>
<tr>
<td>Pitstop-1 (clathrin inhibitor)</td>
<td>1</td>
</tr>
</tbody>
</table>

b) If you assume that the glucose uptake is wholly dependent upon the levels of the active cell-surface insulin-receptor what can you conclude about how the insulin-receptor is endocytosed? (5 marks)
en an initiator caspase and an effector caspase (4 marks).

13. a) Considering mono-ubiquitination only, how many ubiquitins could potentially be added to a protein with the following composition (where the number refers to the number of each amino acid)? (1 mark)

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Arg</th>
<th>Glu</th>
<th>Ile</th>
<th>Val</th>
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</thead>
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<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>19</td>
<td>5</td>
<td>13</td>
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<tr>
<td>Pro</td>
<td>10</td>
<td>Asn</td>
<td>Phe</td>
<td>Met</td>
<td>Trp</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11</td>
<td>8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>2</td>
<td>Cys</td>
<td>Gly</td>
<td>Ser</td>
<td>Ile</td>
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<tr>
<td></td>
<td>12</td>
<td>40</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>4</td>
<td>Asp</td>
<td>His</td>
<td>Thr</td>
<td>Leu</td>
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<tr>
<td></td>
<td>20</td>
<td>9</td>
<td>3</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

b) If a mutation in a cell caused the rate deubiquitination to be higher than ubiquitination, what would be the consequences with regards to

i) mitochondria (2 marks)

ii) Epidermal growth factor receptor (2 marks)

14. How do cytotoxic T-cells and the Fas receptor defend against influenza? (5 marks)
15. The following hydropathy plot indicates that a plasma membrane protein has 7 transmembrane regions and experimentally you know that the c-terminal tail of the protein is ubiquitinated.

a) Is the N-terminus of the protein located extracellularly or in the cytoplasm? Explain your answer. (2 marks)

b) The protein is heavily glycosylated. Would you expect the hydrophilic region on the diagram (approximately amino acids 90-105) to have glycosylation (assuming an abundance of asparagine and/or arginine residues)? Explain your answer. (2 marks)

c) Upon binding an extracellular ligand the protein is ubiquitinated and endocytosed. Where is the likely site of degradation? Explain your answer. (2 marks)

16. a) Name two physiological initiators of autophagy. (2 marks)

b) 3-methyladenine (3-MA) is an inhibitor of autophagy. Give one potential outcome of an impairment of autophagy using 3-MA? (1 mark)
c) You raise the lysosome lumenal-pH by using the V-ATPase inhibitor bafilomycin-A1. What effect would this have on mitochondria and the cell?  
(4 marks)

17. Draw the basic structure of a type I collagen molecule.  
(3 marks)

18. An experimental protocol is being designed to differentiate induced pluripotent stem cells (iPSCs) into corneal tissue to help treat impaired eyesight.
   
a) Provide one advantage and one disadvantage of using iPSCs compared to adult limbal stem cells.  
(2 marks)

b) Part of the protocol involves growing the iPSCs on different extracellular matrix (ECM) substrates, namely type IV collagen and heparan sulfate proteoglycans. Give reasons why these ECM substrates in particular were chosen.  
(2 marks)
c) What in vitro tests would you use to determine if the iPSCs had differentiated into corneal cells? (3 marks)