UNIVERSITY OF YORK
BSc Stage 1 Degree Examinations 2017-18

Department:
BIOLOGY

Title of Exam:
Cell and Developmental Biology

Time allowed: 1 hour and 30 minutes
Total marks available for this paper: 50

- Answer all questions in the spaces provided on the examination paper
- The marks available for each question are indicated on the paper
- A calculator will be provided

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Answer all questions in the spaces provided

1. The SNARE hypothesis explains how the specificity of membrane traffic in eukaryotic cells is achieved. Outline its key features (4 marks).
   Answer: SNAREs are a family of proteins that localize to different intracellular organelles (1 mark). Transport vesicles carry specific v-SNAREs (1 mark) that recognize and bind to t-SNAREs on the appropriate target membrane (1 mark). Membrane fusion can only proceed following formation of a functional SNARE complex (1 mark).

   Relation to Module LO: Describe the organisation and key features of eukaryotic cells. Discuss experimental evidence that supports the key concepts presented in this module.

   Feedback: Generally answered well. The most common reason for dropping a mark was to fail to mention that the SNAREs are a family of proteins and that different intracellular organelles are marked by specific SNAREs.

2. Provide experimental evidence demonstrating that molecular machinery required for membrane traffic in eukaryotic cells is evolutionarily conserved (3 marks).
   Sequence analysis of mammalian NSF and yeast Sec18 revealed homology (1 mark). cDNA encoding NSF (1 mark) complements the growth phenotype of sec18 mutant yeast (1 mark).

   Relation to Module LO: Describe the organisation and key features of eukaryotic cells. Discuss experimental evidence that supports the key concepts presented in this module.

   Feedback: Again this was generally answered well. Incomplete answers only stated the homology between NSF and Sec18 (or between SNAREs from different species) - this in silico analysis alone does not in itself demonstrate the conservation, but rather supports the hypothesis. Other answers that gained, at least partial, marks included supplementing cell free assays of membrane traffic in one species with cytosol from another and showing function. The most common incorrect answer was simply to describe the original cell free assay that identified NSF.

3. a) Provide a piece of evidence that indicates a close relationship between Choanoflagellates and animals. (1 mark)
   Answer: Genome analysis indicates a close relationship between animals and choanoflagellates. Their genome contains many genes found in animals, including genes coding for components of signalling pathways.
Relation to Module LO: Explain how the properties of eukaryotic cells have allowed the evolution and development of multicellular organisms.

Feedback: Generally answered well.

b) Provide a piece of evidence that indicates cell-to-cell signalling predates the evolution of multicellularity. (1 mark)

Answer: The most obvious answer from the lecture material is that single celled eukaryotes like yeast use secreted cell signals to signal mating-type during sexual reproduction.

Relation to Module LO: Explain how the properties of eukaryotic cells have allowed the evolution and development of multicellular organisms.

Feedback: Some answers simply repeated the answer to part A). I only gave 0.5 mark credit for this as the mating type signalling in yeast is a more clear cut answer. Some answers pointed towards yeast mating type but failed to use the key word “signal”. Again, only attracted 0.5 marks for this. Also, some answers alluded to cAMP in aggregation of slime molds. I understand where you are coming from, but in my view slime molds are multicellular organisms, although their strategy for development of multicellularity is different from that in animals, plants, fungi etc.

4. a) How does the mosaic theory of animal development conflict with the principle of nuclear equivalence? (2 marks)

Answer: The mosaic theory of development proposes that cell lineage restriction is dependent on unequal division of nuclear determinants during cell division and differentiation (1 mark). Whereas the nuclear equivalence principle states that during development all cells inherit the same genetic information (1 mark).

Relation to Module LO: Describe the mechanisms regulating eukaryotic gene expression

Feedback: Nuclear equivalence was mostly described well, although I wanted some reference to genetic information rather than “amount of DNA”. Most answers on the right track with mosaic development. However, to get full credit I wanted some reference to unequal division of determinants in cell division and differentiation.

b) Briefly describe an experiment that supports the principle of
nuclear equivalence during animal development. (2 marks)

Answer: The most obvious experiments are animal cloning experiments using nuclear transfer from differentiated somatic cells. A brief and accurate description of Gurdon’s frog cloning experiments or the cloning of Dolly the sheep will attract full marks. An accurate description of the sea urchin cell separation experiments of Driesch would also attract full marks. Also some description of the conclusion that can be drawn.

Relation to Module LO: Discuss experimental evidence that supports the key concepts presented in the module.

Feedback: most answers on track but to get full marks the answer needed to be an accurate description and referenced to a particular species. Some answers were too generic, “divide an embryo”, without referring to sea urchin or somatic nuclear transfer without reference to a species e.g. sheep or frog. I also wanted a brief conclusion about what the experiment tells us.

5. a) Describe how the ras protein functions as a self-regulating molecular switch? (2 marks)

When activated ras, cycles from its inactive GDP bound form to its active GTP bound form (1 mark). In its GTP bound form ras has a GTPase activity which rapidly hydrolyses the bound GTP to GDP. Thus ras is able to self-regulate its own activity (1 mark).

Relation to Module LO: Explain and illustrate with specific examples the general principles that underpin cell signalling in multicellular organisms.

Feedback: the common misconceptions here are that ras is phosphorylated and that it has kinase activity. Quite a few answers understood the basic principle that ras cycles from a a GTP to a GDP bound form but were somewhat vague or inaccurate on how the cycling is achieved. To get full credit some reference need to be made to ras’s GTPase activity. I didn’t think reference to accessory proteins like GEFs and GAPs was strictly necessary.

b) How does activation of the raf enzyme lead to changes in gene transcription? (3 marks)

raf is a kinase that activates the mek enzyme by phosphorylation, which in turn phosphorylates and activates MAP kinase ERK (1 mark). ERK is able to translocate to the nucleus where it phosphorylates and modulates the activity of transcription factors (1 mark). This affects
the ability of these transcription factors to regulate gene transcription (1 mark).

Relation to Module LO: Explain and illustrate with specific examples the general principles that underpin cell signalling in multicellular organisms.

Feedback: most answers had an accurate description of the MAP kinase cascade, although I wanted to see reference to “phosphorylate and activate”. Some answers only said one or the other. Quite a lot of answers made the very common mistake of thinking Erk is a transcription factor that regulates transcription directly. The mistake meant that the answer could not get more than 1.5 marks. I wanted a clear understanding that Erk can go to the nucleus and phosphorylate transcription factors which then alter gene transcription.

6. You have identified a novel protein closely related to nodal in the frog Xenopus. How would you investigate if this protein can function as a morphogen? (2 marks)

Needs some acknowledgement that a morphogen is a factor that elicits specific effects on a cell in a concentration dependent manner (1 mark). The most obvious example from the lectures would be to test if different concentrations of this protein could induce different types of mesoderm in the cells of animal cap explants (1 mark).

Relation to Module LO: Interpret experimental evidence and devise simple, testable models based upon this evidence.

Feedback: Any answer that made reference to concentration or gradient attracted 1 mark. There were a wide range of experiments suggested, some practical, some not so practical. However, if reference was made to some differential cellular response then this got another 0.5 marks. Many answers fell into this category. However, given the reference to nodal I thought it was not unreasonable to make some reference to mesoderm, and this was required to get full marks. Pleasingly quite a few did get full marks for this.

7. Compare and contrast how cortisol and retinoic acid signalling lead to activation of gene transcription. (4 marks)

Both cortisol and retinoic acid signal through nuclear hormone receptors. In the case of cortisol signalling, in the absence of the ligand the receptor is held in an inactive complex in the cytoplasm (1 mark). In the presence of cortisol the receptor is released from
the complex and translocates to the nucleus where it can bind to DNA and regulate the expression of target genes (1 mark). In contrast, in the absence of retinoic acid the RA receptor is already bound to target genes in association a transcriptional repression complex (1 mark). In the presence of RA, the transcriptional repressor is exchanged for a transcriptional activator leading to the activation of transcription from the target genes (1 mark)

Relation to Module LO: Explain and illustrate with specific examples the general principles that underpin cell signalling in multicellular organisms.

Feedback: lots of well constructed and very accurate descriptions here, highlighting the similarities and differences. A recurring inaccuracy was confusing cortisol and RA with their receptors. Cortisol and RA are not transcription factors but the ligands that bind to nuclear hormone receptors. The other slight confusion was the idea that on binding of the RA the receptor moved to different cis-regulatory elements rather than exchanging co-repressors for co-activators in the bound receptor complex. I marked this down a bit.

8. a) What are the three main classes of cis-regulatory elements? (3 marks)

i) ii) iii) Promoters, enhancers and silencers

Feedback: Unsurprisingly quite a few people aced this. More surprising, was that some students appeared to have no clue at all and simply answered with three random phrases from the module.

Relation to Module LO: Describe the mechanisms regulating eukaryotic gene expression

b) What are the functions associated with general transcription factors and where within the cis-regulatory regions of protein coding genes do they bind? (3 marks)

General transcription factors are components of the transcription initiation complex, containing RNA pol II, that is assembled at the promoters of all protein coding genes (1 mark). RNA polymerase II is unable to transcribe a gene on its own (1 mark). General transcription factors have various
activities that enable RNA polymerase II to function in gene transcription including TATA box recognition, recruiting RNA pol II and helicase activity (1 mark).

Relation to Module LO: Describe the mechanisms regulating eukaryotic gene expression

Feedback: Generally answer well. I withheld marks if there was no acknowledgement of the fact that GTFs are required for the transcription of all protein coding genes. I also wanted some mention of a function other than recruiting RNA pol II.

c) With reference to a specific example, describe how trans-acting factors are able to regulate cell lineage. (5 marks)

The example that will likely be used will be the regulation of the skeletal muscle lineage by myogenic regulatory factors, such as MyoD etc (1 mark). These are bHLH transcription factors expressed exclusively in the cells that will develop as skeletal muscle (1 mark). They activate transcription of genes coding for proteins required for muscle development e.g actin, myosin, tropomyosin (1 mark). They regulate transcription by binding as a dimer with the E12 protein to E-box sequences in the cis-regulatory of these genes (2 marks).

Relation to Module LO: Describe and illustrate with specific examples the mechanisms regulating cell growth, cell death and cell-type specification in multicellular organisms.

Feedback: This question was quite a discriminator. Most answers had an appropriate statement about transcription factors binding to cis-regulatory elements and regulating cascades of gene expression leading to lineage restriction. Disappointingly, many of these answers then went to discuss signal transduction pathways. These answers did not attract any marks unless the answer also addressed the specifics of transcriptional regulators at the end of these pathways. Quite a few answers discussed the role of EPO in blood cell lineage specification. EPO is a not a transcription factor...Others did address some of the transcription factors e.g. FOG in this lineage and this did attract some marks. The obvious choice however, was Myod. There was plenty to talk about from lectures on mechanism of action. Disturbingly, some answers correctly identified MyoD as being relevant but then discussed its mode of action binding to cell surface receptors of the TGF-beta pathway.

9. Briefly explain how you could test the ability of haematopoietic stem cells to differentiate into red blood cells (erythrocytes). (4 marks)
Not directly taught. In vitro: Should understand that the HSCs should be exposed to a specific induction stimulus, erythropoietin (EPO) (1) in this case. Could reference how the HSCs will progress through different intermediate stages (BFU-E and CFU-E) to form mature erythrocytes (1). Differentiation status should be tested (1), stating how (1) (e.g. using specific cell surface markers for RBCs as well as loss on HSC marker expression, haemoglobin expression, even morphology, loss of nuclei).
Alternatively, the more rigorous assays will be by in vivo implantation (1) into a radiated mouse model (1) and determine restoration of circulating, differentiated (1) functional RBCs (1).

**Relation to Module LO:** Describe the organisation and key features of eukaryotic cells. Describe and illustrate with specific examples the mechanisms regulating cell growth, cell death and cell-type specification in multicellular organisms.

**Feedback:** There were some good answers but marks were lost when the specifics of the question were not addressed, primarily to test differentiation into erythrocytes (rather than all haematopoietic cells). So many answers described the in vivo functional repopulation experiment from the lectures, without explaining how erythrocyte presence/function would be tested.

10. An experiment was set up to determine changes in protein expression during the cell cycle. Epidermal stem cells were grown in Petri dishes in culture medium that was supplemented with serum to generate sufficient cells for the experiment. Serum contains many growth factors and cytokines to promote cell division. At 24 hours before the start of the experiment, the epidermal stem cells were switched to a culture medium without serum.

The serum was then replaced and protein samples were taken from the epidermal stem cells every 2 hours for 14 hours. The protein samples were analysed by SDS-PAGE and are shown in the figure below.
Figure legend: SDS-PAGE analysis of protein expression in epidermal stem cells during the cell cycle. After 24 hours of serum-starvation, serum was added back to the cell cultures and protein extracted every 2 hours for 14 hours and loaded onto the gel (lanes 1-7). The gel was stained to allow visualisation of the different sized proteins.

a) Where are epidermal stem cells normally located in the body? (1 mark)

In the basal layer, or the bulge region, in the epidermis of skin. Cannot accept just “skin”, needs to be specific.

Feedback: Mostly correct, but as in the model answers, no marks if too vague.

b) Why was serum first removed from the epidermal stem cells for 24 hours and then added back at the start of the experiment? (2 marks)

Example marks: Serum contains factors that allow the continued growth of cells and the cells will be at different stages in the cell cycle, making the interpretation of changes in protein expression during the cell cycle difficult (1 marks). Serum-starvation (removing serum) means the cells will not proliferate and will synchronise in G0 (1). Serum is returned to the culture to initiate cell cycling simultaneously across the cell population (1).

Feedback: Many full, correct answers. Answers need to address both parts of the question; why serum was removed and why it was added back.
c) The SDS-PAGE analysis suggests that the expression levels of one protein clearly changes as the cells progress through the cell cycle. On the figure above, label this protein as “Protein A” with an arrow (1 mark).

Answer:

![SDS-PAGE Image]

Feedback: Most correct. Marks were lost if the arrow singled out one band only, as the gel shows protein A across the lanes changing expression over time.


d) In the cell cycle of epidermal stem cells, it is known that M phase lasts about 1 hour, the G1 phase lasts approximately 3-4 hours, S phase lasts approximately 7-8 hours and G2 around 4-6 hours. Referring to the SDS-PAGE results, what can you deduce about the expression of Protein A in these epidermal stem cells during the cell cycle? (4 marks)

At the start of the experiment (serum addition), the cells will enter the cell cycle at G1 (1 mark). In G1 (lane 1), protein A levels are low/absent (1). G1 lasts 3-4 hours, so lane 2 may represent late G1 or early S phase and Protein A levels begin to increase to reach a peak around 8 hours (lane 4) during S phase (1). S phase will last 7-8 hours, so from lanes 3-6 (6-12 hours after the start of the experiment) (1). After this time, Protein A levels reduce (lane 7) (1). Protein A expression is associated with the S phase of the cell cycle (1).

Feedback: The data were interpreted well in general and good answers were given based on cell cycle understanding. It was important to explain the answers in relation to the SDS-PAGE results. From the results it is not possible to conclude that Protein A is a Cyclin, though this can be hypothesised.

Relation to Module LO: Interpret experimental evidence and devise simple, testable models based upon this evidence. Describe and illustrate with
specific examples the mechanisms regulating cell growth, cell death and cell-type specification in multicellular organisms.

11. Using three examples, compare and contrast endochondral and intramembranous ossification (3 marks).

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<td>1  Long bone development</td>
<td>Flat bone development</td>
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<td>2  Originates from mesenchymal condensation</td>
<td>Originates from mesenchymal condensation</td>
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<td>3  Develops through cartilage model/intermediate (indirect)</td>
<td>Direct formation of bone (not via cartilage)</td>
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<td>4  Requires the transcription factor, Runx2 for osteogenesis (and Sox9 for chondrogenesis).</td>
<td>Requires the transcription factor, Runx2. No chondrogenesis.</td>
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<td>5  Involves vascular invasion.</td>
<td>No vascular invasion.</td>
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Relation to Module LO: Explain how the properties of eukaryotic cells have allowed the evolution and development of multicellular organisms.

Feedback: Many full-mark answers, some quite inventive, which were also scored fully if correct. The main reasons for losing marks was overlap - two answers being too similar to each other; or the answers were vague and/or did not directly compare the two processes (e.g. “indirect” vs “Requires Runx2”).