Module Code: BIO00052I

Examination Number: ____________

BSc Degree Examinations 2019-20

Department:
BIOLOGY

Title of Exam:
Molecular genetics and development

Time Allowed:
24 Hours (PLEASE NOTE: Late papers will not be marked)

Time Recommended:
2 hours and 30 minutes

Word Limit: Please answer the questions within 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:
All questions should be answered on this question paper using minimum font size Arial 11.
Each question should be answered within the stated line limit.
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. a) Outline the hierarchy of gene expression activated downstream of the maternally deposited protein Bicoid that results in the patterning of 14 parasegments in the Drosophila embryo. (9 marks, 25 lines)

Maternal: When the Drosophila egg is released from the ovary there is already patterning info laid down by the mother (such as the maternal Bicoid gradient). (1 mark)

Zygotically acting segmentation genes (gap genes, pair-rule genes, and segment polarity genes) interpret maternal gradients through successive levels of gene hierarchy(1 mark).

Some of these genes (such as bicoid) code for transcription factors that can act as morphogens, which is unusual, but can happen because the early fly embryo is a syncitium(1 mark).

Zygotic: GAP genes: The maternal gradient of bicoid results in the activation of Gap gene expression in broad overlapping domains(1 mark) along the AP axis. Cells are allocated to large contiguous sections along the AP axis by Gap genes; Mutations in Gap genes results in the loss of these large domains(1 mark).

Pair-rule genes: Later, cells are allocated to 14 parasegments (1 mark): this happens through the action of pair-rule genes (like ftz and eve) that are expressed in response to Gap genes. Mutations result in a loss of alternate PS(1 mark)

Segment polarity genes: Subsequently the parasegments are patterned along the A/P axis by segment polarity genes(1 mark) (like engrailed, wingless and hedgehog) that are expressed in response to pair rule genes. A strict PS boundary(1 mark) is set by the activity of these genes. Mutations result in a loss of AP patterning within a PS (1 mark)

b) Give a piece of evidence for one level of the gene hierarchy you describe in (a). (2 marks, 4 lines)

Some acceptable answers (not limited to these):

- Stripe 2 of the pair rule gene eve is regulated by an enhancer with overlapping binding sites for Gap gene transcription factors that act as activators (including Hb) and repressors (including Gt, Kr)
- In a pair rule mutant (ftz) the segment polarity gene engrailed isn’t expressed in alternate stripes, while an engrailed mutant doesn’t affect ftz expression.
- Overexpression of bicoid expands the anterior domain of the gap gene hunchback
2.

a) Describe the methods you would use to determine whether the gene coding for sonic hedgehog (Shh) is transcribed in mouse limb bud.  
   (1 mark, 3 lines)

   use in situ hybridisation (1 mark) (or will accept qPCR on dissected tissue for part credit) to determine localisation of mRNA using a labelled, complementary (antisense) probe.

b) How would you determine whether the Shh protein is co-expressed with the mRNA?  
   (2 marks, 4 lines)

   Carry out immunohistochemistry using an antibody (1 mark) raised against the shh protein; use a fluorescently (or otherwise) conjugated secondary antibody to visualise expression. Compare these findings with data from in situ hybridisations. (1 mark)

c) What is the evidence that mutations within the ZRS (Zone of polarizing activity Regulatory Sequence) has resulted in limblessness in advanced snakes?  
   (5 marks, 15 lines)

   The ZRS is a conserved enhancer that drives Shh expression in the ZPA in limbed vertebrates. (1 mark)

   Changes to the sequence of this enhancer in snakes means it is less/not active in snakes (1 mark)

   More changes in this sequence in advanced snakes which are completely limbless. (1 mark)

   Reporter with human ZRS in mice drives normal ZPA expression, python ZRS is weaker, transient (1 mark)

   Using gene editing, the normal mouse ZRS enhancer can be replaced with the ZRS of a snake resulting in a 'serpentized' limbless mouse. (1 mark)

d) What is the evidence that Shh acts as a morphogen to pattern the ventral neurons in the vertebrate neural tube?  
   (3 marks, 9 lines)

   Shh mRNA is transcribed and translated in cells in the floorplate of the neural tube (and notochord); the protein is secreted and diffuses away from the floorplate creating ventral-to-dorsal gradient of shh protein (IHC). (1 mark)

   This acts as a morphogen to pattern the different cell type in the NT. (1 mark)
Explants of naïve neural tissue treated with different concentrations of Shh protein are measured for what neural markers they express. The highest levels of Shh give more ventral type neural markers. These types of neurons are closest to the endogenous source of Shh suggesting it acts as a morphogen in vivo. (1 mark)

3.

a) What is the evidence that neural crest cells are pluripotent? (2 marks, 6 lines)

Neural crest cells give rise to multiple cell types (1 mark) and tissues including bones of the face and head, neurons the peripheral nervous system and gut, and all the melanocytes.

NCCs maintain a pluripotent state by expressing pluripotency genes including the Oct and Sox genes. (1 mark)

b) Some labs investigating the neural crest are funded by cancer charities; what is the justification for this? (3 marks, 8 lines)

EMT is the cell behaviour underlying neural crest delamination from the dorsal neural tube. (1 mark)

In cancer progression, EMT is triggered in malignant cells as they begin to migrate to form metastatic cancers. (1 mark)

Understanding the mechanism/genes regulating EMT in model organism like a chick, leads to a better understanding of invasive cancers. (1 mark)

c) Neural crest cells migrating via the ventral pathway only travel through the anterior half of the somite scleratome. What is the molecular basis of this? (4 marks, 10 lines)

Ephrins are expressed in the posterior half of the somite scleratome. (1 mark)

The neural crest cells recognize the ephrin proteins through their cell surface Eph receptors. (1 mark)

Binding to the ephrins activates the tyrosine kinase domains of the Eph receptors. (1 mark)

This results in a repulsive cue in NCCs, likely interfering with the actin cytoskeleton that is critical for cell migration. (1 mark)
4.

a) With reference to specific examples, describe the evidence that metazoan evolution was accompanied by genomic innovation associated with cell signalling and transcriptional regulation. (3 marks, 9 lines)

Answer:
- Comparative analysis of metazoan genomes and nearest unicellular ancestors reveals that the evolution of metazoans was accompanied by the evolution of multiple novel gene families associated with these processes (1 mark).
- Transcriptional regulation: Hox-like, Pou domain and Ets transcription factors (naming at two families will attract a full mark)
- Cell signalling: TGF-β, wnt ligands, nuclear hormone receptors (naming at two families will attract a full mark)

You have identified a novel species of segmented, bilaterally symmetrical animal living in a deep trench in the Pacific ocean. Your analysis of gut development in this organism reveals the presence of a central through gut, with a posterior opening that forms during gastrulation and an anterior mouth opening that forms later in development.

b) Do you think this organism is more closely related to the chordate or arthropod lineages? Explain your answer. (2 marks, 5 lines)

Answer: the development of the posterior opening of the gut before the anterior opening is typical of deuterostome embryos (chordates), rather than protostomes (arthropods) where the mouth opening develops first. It is therefore likely to be more closely related to chordates (2 marks)

c) Name two other anatomical features of the developing embryo that could be examined to help confirm your conclusions (2 marks, 4 lines)

Answer: Two from presence of look for the presence of dorsal nerve tube, presence of dorsal notochord, presence post-anal tail, segmental muscle blocks. Would also accept the presence of ventral circulatory pump.
Based on an analysis of the organism’s genome, you have identified and analysed the mRNA expression patterns of many developmentally important genes, including *bmp4*, *brachyury*, *chordin*, the *hox* genes and *distalless*.

   d) The expression patterns of which of these genes would you consider to be most helpful in supporting your conclusion to part a)? With reference to the predicted expression patterns, explain your reasoning. (3 marks, 8 lines)

Answer: *bmp4* and *chordin* (1 mark). These two genes have conserved functions in regulating development of the dorso-ventral axis of all bilaterally symmetrical animals (1 mark). However, due to axis inversion between protostomes and deuterostomes they are expressed on opposite sides of the embryos. Based on the available data this animal is a deuterostome and the prediction would be that chordin would be expressed on the dorsal side of the embryo and BMP4 on the ventral side (1 mark).

*NB The answers to parts c) and d) are somewhat contingent on the answer to part b). However, as long as the answers to part c) and d) are consistent with that to b) they will attract marks."

Relation to MLOs:
- Describe the molecular genetic and epigenetic mechanisms regulating development.
- Interpret experimental evidence related to gene function, epigenetic regulation and genetic disorders.
- Understand how a toolkit of techniques can be used to investigate gene function, and be able to apply this knowledge to design rationale experimental approaches to answer problems related to development of multicellular organisms.

5.

   a) What do you understand by the term colinear Hox gene expression? (2 marks, 5 lines)

Answer: Where and when a particular Hox is expressed reflects its position in a given Hox cluster (1 mark). Genes from the 3’ end of a Hox cluster are expressed earlier in development and in more anterior regions than genes from the 5’ end of a cluster (1 mark).
b) With reference to a named example, discuss the evidence that a change in Hox protein sequence and function is associated with changes in animal body plan.  

(8 marks, 20 lines)

Answer: Distalless expression is required for limb outgrowth in arthropods and is only expressed in segments anterior to the abdomen (1 mark). Insect abdA and Ubx are expressed in the abdomen and are required for the repression of distalless expression in the abdomen (1 mark). The importance of this repression is shown by the formation of ectopic legs in the abdomen of flour beetle double mutants for abdA and Ubx (1 mark).

In contrast, crustacean have many more legs than insects, and Ubx and abdA are expressed in the leg forming region (1 mark). Therefore, it would appear that the crustacean hox proteins do not inhibit distalless expression. This is further supported because the ectopic expression of crustacean abdA in insects does not inhibit distalless expression. The same is also true of AbdA from the primitive many legged arthropod onychophora (2 marks).

Acquisition of the ability of Ubx and Abd-A in insects to repress distalless is associated with the development reduced number of appendages the hexapod body plan of insects compared to crustaceans and more primitive arthropods (2 marks).

Relation to MLOs:
- Describe the molecular genetic and epigenetic mechanisms regulating development.

6.

a) Describe the evidence indicating the presence of a nodal signalling gradient in the early amphibian embryo.  

(2 marks, 4 lines)

Answer: In situ hybridisation shows that nodal mRNA is expressed in dorsal to ventral gradient in the vegetal hemisphere (1 mark). Western blot analysis shows that there are higher levels of phospho-Smad2 on the dorsal side of the embryo (1 mark).

b) Describe a piece of evidence indicating that cells from the amphibian embryos exhibit threshold responses to the nodal signalling gradient.  

(2 marks, 4 lines)

Answer: Analysis of gene expression in animal cap explants reveals that brachyury is maximally expressed at low levels of nodal signalling and is inhibited
at high levels, whereas gsc requires a higher threshold of nodal signalling for maximal expression (2 marks).

c) Briefly describe how you might make a reporter gene construct that responds to nodal signalling.  

Answer: Clearly lots of possibilities. An obvious approach would be to take the promoter and cis-regulatory elements of a nodal responsive gene like brachyury or gsc and attach this to a reporter like luciferase or GFP. Any plausible approach will attract marks.

Relation to MLOs:

● Describe the molecular genetic and epigenetic mechanisms regulating development.

● Understand how a toolkit of techniques can be used to investigate gene function, and be able to apply this knowledge to design rationale experimental approaches to answer problems related to development of multicellular organisms

7. You have identified a novel drug that binds to amphibian notch receptors. Your hypothesis is that the drug will activate notch signalling in the cells of the embryo in the absence of the delta ligand. Design and justify an experimental approach to test this hypothesis.

Answer: There are several possibilities, but based on the material presented, the most obvious approach would be to investigate the effects of the drug on neuronal differentiation during neurogenesis in the amphibian neural plate. As predicted by the lateral inhibition model of neurogenesis and experiments using a constitutively active notch receptor, activation of notch signalling inhibits proneural gene expression and neuronal differentiation (2 marks). The experiment would then be to treat open neural plate stage embryos with the drug. Proneural gene or neural differentiation makers could then be examined by in situ hybridisation (2 marks). If the hypothesis is the expression of these genes will be inhibited by the drug (1 mark).

Relation to MLOs:

● Describe the molecular genetic and epigenetic mechanisms regulating development.

● Understand how a toolkit of techniques can be used to investigate gene function, and be able to apply this knowledge to design rationale experimental approaches to answer problems related to development of multicellular organisms
8. Summarize and critique the experimental approach used by Kass et al. (1997) that demonstrated a link between DNA methylation and repression of gene expression.

(5 marks, 10 lines)

Reporter DNA constructs were injected into Xenopus oocytes. The CAT reporter gene was under the control of the HSV tk promoter that was either methylated or unmethylated. RNA samples were prepared from the injected oocytes over a time course and analyzed for expression of the reporter gene. A control consisting of a different injected construct that was always unmethylated was included. Could argue that this is not representative of the endogenous process as the promoter was in vitro methylated and this may be beyond that occurring naturally. Also the analyzed genes are injected rather than being part of the chromosome and so repressive processes may be different. Most students could summarize the experimental approach however the critique was missed by many. Valid criticisms that differ from the model answer were credited. A minority of students misunderstood the experiment and suggested that the experiment was aimed at detecting the appearance of DNA methylation over time.

9. Experiments were performed to investigate epigenetic marks that are associated with CpG islands (Figure 1). DNA samples were prepared from mouse brain nuclei using a technique that ensures that any associated proteins remain attached. DNA samples consisted of either non-methylated CpG islands (CGI) or total DNA (Bulk). Antibodies specific to different histone modifications and DNA binding proteins (Cfp1 and Kdm2a) were then used to assess the extent to which they were associated with the two different DNA samples. Equal amounts of DNA were analyzed in each case.

Figure 1.
a) Why do you think that the researchers used antibodies against Histone H3 that do not distinguish between different modifications in their experiment?

As a control to check whether equal amounts of histone proteins are present in the two samples. Most students suggested it was a control but few could accurately describe what it was actually controlling. Many vague answers here.

b) What do you conclude from this experiment? To what extent are the results for the histone modifications expected?

That compared with total DNA, CpG islands are enriched for Acetylated H3, H3K4me3 and H3K4me2 (all histone marks associated with open/active chromatin). Compared to bulk DNA they have few repressive marks such as H3K9me3 and H3K29me3. They are also enriched for the DNA binding proteins Cfp1 and Kdm2a which suggests they bind to CpG island sequences. Results are expected as most CpG islands, being generally free of DNA methylation, would be expected to not have repressive histone marks and be enriched for active marks. Bulk DNA would be expected to be a mixture of active and repressed chromatin and so results fit with expectations.

Most scored well on this question.

c) Describe an experimental approach that would allow you to confirm the findings of the experiment shown in Figure 1.

Via ChIP with antibodies against the proteins used in Figure 1 to evaluate whether Acetylated H3, H3K4me3 and H3K4me2, Cfp1 and Kdm2a are enriched at CpG islands compared to other genomic regions. Would need to produce a genome-wide profile and compare distributions or PCR for specific regions. Most scored well though some suggestions did not actually confirm the findings and so marks were lost for not addressing the question.

MLO: Describe the molecular genetic and epigenetic mechanisms regulating development.

Interpret experimental evidence related to gene function, epigenetic regulation and genetic disorders.

10.

a) In the absence of Nodal signalling, histones associated with Nodal target genes have the repressive H3K27me3 modification. Explain how H3K27me3 is removed from Nodal targets upon Nodal signalling and describe the supporting experimental evidence.
Nodal signalling will activate Smad2/3 which are able to recruit the Jmjd3 histone demethylase to Nodal targets that is active against H3K27me3 (1). Supporting evidence includes ChIP experiments demonstrating the presence of Jmjd3 at Nodal targets that is significantly reduced when cells are treated with inhibitors of Nodal signalling (1). Inhibition of Nodal leads to higher levels of H3K27me3 than in the absence (1).

Most scored well on this question with marks lost for loss of detail or missing out the ChIP experiment with Jmjd3.

b) How could you test whether Nodal signalling triggers a change in DNA methylation at Nodal target promoters? (2 marks, 4 lines)

Perform bisulfite sequencing on samples derived from cells in the absence and presence of a nodal inhibitor (1). Bisulfite treatment is followed by PCR for the target promoters and then sequencing (1)

A common mistake was to suggest ChIP here and this showed that some students had confused DNA methylation with histone methylation. However most did correctly suggest bisulfite sequencing.

MLO: Describe the molecular genetic and epigenetic mechanisms regulating development.

Interpret experimental evidence related to gene function, epigenetic regulation and genetic disorders.

11. Briefly summarize the changes in DNA methylation that occur in early mammalian embryonic development and discuss why these changes are necessary. (10 marks, 20 lines)

There is a large-scale erasure of DNA methylation that is faster for the paternal genome than the maternal and occurs immediately post-fertilization (1). The paternal genome has a higher methylation level than the maternal and erasure may be required to reset developmental potential allowing expression of pluripotency genes such as Oct4 in the early embryo (1) and possibly to remove any accumulated DNA methylation changes (1). This erasure effectively removes parent-of-origin methylation marks though some sequences escape including imprinted genes and many transposable elements (1). This ensures that the balance of gene expression for imprinted genes is maintained - loss of this can be lethal or cause significant phenotypic problems (1). The low point of methylation is around the blastocyst stage and from this point the genome becomes re-methylated in a global pattern apart from CpG islands (1). Primordial germ cells destined to give rise to the gametes undergo a second wave of demethylation (1)
to remove the somatic methylation pattern and replace it with a sex-specific pattern that enables imprinted gene expression in the next generation (1). Some CpG islands become methylated in a tissue-specific manner in the developing embryo, these are usually at key regulatory genes that no longer need to be expressed (1). In female mammals DNA methylation changes on the inactive X chromosome will take place as part of the repression (1) Mostly good answers though the discussion of why the changes are necessary was missing in some. A common mistake was to focus heavily on the mechanistic detail which was not actually asked for in the question. Some answers only focused on the demethylation step post-fertilization and so didn’t give a full answer.

MLO: Describe the molecular genetic and epigenetic mechanisms regulating development.

Interpret experimental evidence related to gene function, epigenetic regulation and genetic disorders.

12. Mind bomb (MIB) is a ubiquitin ligase which is essential for functional Notch signalling. It ubiquitinates Notch receptors (such as JAG1) and is essential for their function.

In the experiment below, mRNA encoding four mutant forms of MIB found in human patients with cardiac defects were over expressed in zebrafish embryos together with Flag-tagged JAG1 and HA-tagged Ubiquitin. An immunoprecipitation was performed using anti Flag antibodies to assess the levels of ubiquitination of JAG1.
a) Explain the results of the experiment. (4 Marks, 9 lines)

Immunoprecipitation with anti flag pulls down the JAG1 ligand and the labelled ubiquitin with it (1 mark). Overexpression of Wild type MIB causes strong ubiquitination of JAG1 compared to vector control (1 mark). p.F520R produces similar levels (1 mark) but all the other mutants cause far less ubiquitination of JAG1 (1 Mark)

b) The mRNA encoding for the p.T312L fs*55 mutant protein causes the least ubiquitination of JAG1 in this experiment. Why do you think this is? (2 Marks, 5 lines)

T312L fs*55 is a frameshift mutation (designated by the fs*55) but the other mutations are mis-sense mutations. (1 Mark). Hence it probably causes an early premature stop leading to a truncation of the protein and a more severe effect on its function (1 Mark).

c) These MIB mutations are thought to cause their phenotype due to their effect on the cardiac neural crest. Name two tissues that the cardiac neural crest contributes to. (2 Marks, 3 lines)

Any two from: Outflow tract septum (1) Aortic arch arteries (1) Smooth muscle around aortic arteries (1) Valves (1) cardiac septum (1) Thyroid (1) Parathyroid (1)

d) Briefly describe some of the evidence for why we think the neural crest contributes to the development of these tissues. (2 Marks, 5 lines)

Lineage studies in Chick Quail Chimeras show contribution from the grafted cardiac neural crest lineage to these areas (1) Cardiac neural crest ablation studies lead to defects in these areas (1)

MLO:
Describe the molecular genetic and epigenetic mechanisms regulating development. Describe how knowledge of the conserved mechanisms regulating development has been important for understanding the genetic basis of some human diseases. Interpret experimental evidence related to gene function, epigenetic regulation and genetic disorders.

End of Exam