BSc / MSc Degree Examinations 2018-9

Department:
Biology

Title of Exam:
Molecular genetics and development

Time Allowed:
2.5 hours

Allocation of Marks:
Marks for each questions are indicated

Instructions for Candidates:
All all questions in the space provided

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. a) Describe, or draw, what a Drosophila embryo transgenic for En::LacZ looks like after staining for beta-galactosidase. Explain this observation. (6 marks)

14 blue stripes. (2 marks) En::LacZ is a reporter that shows the expression of engrailed, a segment polarity gene. The En::LacZ reporter has the regulatory region/enhancer of engrailed upstream of LacZ, so that the cells that express engrailed will activate lacZ, which codes for beta galactosidase which turns a substrate blue. In embryos, engrailed is expressed in 14 stripes; so the En::lacZ transgenic embryos display 14 blue stripes after staining for beta-galactosidase. (4 marks)

b) When generating transgenic flies, why are plasmids injected into the posterior cytoplasm of the egg? (4 marks)

The pole cells form in the posterior of the egg (1 mark) and will give rise to the germ line (1 mark). The plasmids need to be taken into the nuclei in these pole cells in order to be passed onto the next generation (1 mark) to generate transgenic flies in the offspring of the injected flies (embryos) (1 mark).

2. a) Describe the methods you would use to determine when and where in the embryo the gene coding for sonic hedgehog is expressed (transcribed). (2 marks)

Collect and fix embryos at a range of stages (0.5 mark) during development and use in situ hybridisation (1 mark) (or will accept qPCR on dissected tissue for part credit) to determine localisation of mRNA (.5 mark) using a labeled, complementary probe. Protein level expression answers would also be acceptable or use a reporter with regulatory region of Shh upstream of the coding region of LacZ/GFP.

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

Carry out immunohistochemistry (.5 mark) using an antibody (.5 mark) raised against the shh protein; use a fluorescently (or otherwise) conjugated secondary (.5 mark) antibody to visualise expression. Compare these findings with data from in situ hybridisations (.5 mark).

c) Discuss the significance of the different distribution of the Shh mRNA and Shh protein in the ventral part of the neural tube. (3 marks)

Shh mRNA is transcribed and translated in cells in the floorplate of the neural tube (and notochord) (1 mark); the protein is secreted and diffuses away from the floorplate (1 mark) creating ventral-to-dorsal gradient of shh protein. This acts as a morphogen (1 mark) to pattern the different cell type in the NT.
d) what is the evidence that Shh acts as a morphogen to pattern the ventral neurons in the vertebrate neural tube? (3 marks)

Explants of naïve neural tissue (.5mark) treated with different concentrations (.5mark) of Shh protein are measured for what neural markers(.5mark) they express. The highest levels of Shh give more ventral type neural markers (.5 mark). These types of neurons are closest to the endogenous source of Shh suggesting it acts as a morphogen in vivo (1mark).

3 a) Explain the observation that segment polarity genes code for many different types of proteins while gap genes and pair-rule genes code only for one class of protein. (4 marks)

The early drosophila embryo develops as a syncytium with many nuclei sharing the same cytoplasm (1mark). Therefore transcription factors can diffuse among nuclei (1mark), GAP genes code for only for TFs and act early during the syncitial blastoderm stage(1mark). Segment polarity genes act later in development ,after cells form, so code for proteins like receptors and signalling molecules(1mark).

b) Assess the experimental evidence that vertebrate Hox genes are orthologues of the Drosophila homeotic genes. (6 marks)

Good assessment of the following info: like fly homeotic genes, Hox genes are clustered(1mark), expressed co-linearly (3’ anterior, 5’ posterior)(1mark), have strict domains of expression with sharp anterior boundaries(1mark) that align to compartments(1mark), and HoxB4 knock out leads to posterior to anterior transformation(1mark). Should mention gene duplication(1mark).

4. Discuss the anatomical and molecular evidence supporting the theory that the dorsal-ventral axis was inverted during evolution of deuterostomes and protostomes. (5 marks)

Answer: Examination of animal anatomy shows that in both deuterostomes and protostomes there is a central through gut. However, in protostomes there is a ventral nerve cord and dorsal circulatory pump, whereas in deuterostomes the position of these structures is reversed (dorsal nerve cord and ventral circulatory pump (2 marks). Dorsal ventral patterning in all animals is dependent on gradients of BMP signalling and its secreted inhibitor chordin (1 mark). In protostomes, such as insects, the chordin homologue (SOG) is expressed in the ventral region and BMP in the dorsal region. In keeping with axis reversal, in deuterostomes, such as vertebrates, chordin is expressed dorsally and BMP ventrally (2 marks).
5. a) Explain why neural induction and neurogenesis should be considered distinct processes in vertebrate development. (2 marks)

Answer: Neural induction occurs first during development and is a signalling process that defines a neural territory which gives rise to the nervous system (1 mark). Primary neurogenesis occurs after neural induction and selects individual cells within the neural territory to differentiate as neurons in the primary nervous system (1 mark).

b) How do the activities of proneural and neurogenic genes differ in the process of neurogenesis? (2 marks)

Answer: The function of proneural genes is to mark out an ectodermal territory that is competent to form neurons. Proneural genes promote neurogenesis (1 mark). Conversely, the function of neurogenic genes is to limit the number of cells differentiating as neurons in proneural expressing domains. Neurogenic genes inhibit neurogenesis (1 mark).

c) The newly identified drug “Jagactin” has been shown to bind to and activate the notch receptor in cell culture. Briefly describe an experimental approach to investigate whether Jagactin activates notch signalling in developing frog embryos. Explain the rationale for your design. (4 marks)

Answer: The approach suggested by material presented in lectures would be to treat neurula stage Xenopus embryos with the drug and analyse the effects on primary neurogenesis (1 mark). This could be investigated by analysing expression of the neuronal differentiation marker n-tubulin(1 mark). Activation of notch signalling inhibits proneural gene expression. Therefore, it is predicted that if Jagactin activates notch signalling treatment of would inhibit neuronal differentiation as shown by reduced n-tubulin staining (or proneural gene expression)(2 marks).

6. The downstream transcriptional effectors of Wnt signalling are TCF transcription factors, which bind to Wnt response elements with a consensus sequence ATCAANG (where N=A, C, G or T) in the enhancers of Wnt regulated genes. Figure 1A
Expression of the \textit{wix1} gene is upregulated in isolated \textit{Xenopus} embryonic cells in response to wnt8 protein. To study the transcriptional regulation of \textit{wix1}, a region of the gene upstream of the transcriptional start site was isolated and sequenced (Figure 1A). This was used to make a \textit{wix1} luciferase reporter gene (\textit{wix1-luc}) (Figure 1B).

Figure 2

a) What do these data tell us about the \textit{wix1-luc} transgene and the upstream sequence that it contains? (3 marks)

Answer: The temporal expression profile for the endogenous gene is closely matched by that of the reporter transgene (1 mark). This indicates that the reporter recapitulates the expression of the endogenous gene. Therefore, the identified upstream region of \textit{wix-1} within the \textit{wix1-luc} reporter probably contains all the regulatory information necessary to drive the normal expression pattern of this gene (2 marks).

Additional \textit{wix1-luc} derived constructs were produced, in which either bases 1 to 30 (\textit{wix1-D30-luc}) or bases 31 to 54 (\textit{wix1-D54-luc}) were deleted from the upstream region of \textit{wix1}. Figure 3 shows the activity of the wild-type and deletion \textit{wix1-luc} reporters in control
embryonic cells and cells treated with wnt8.

Figure 3

b) Based on the available data, present a hypothesis to explain the observed effects on reporter activity. (4 marks)

Answer: Examination of the sequence 31 to 54 reveals the presence of 2 consensus TCF binding sites for TCF transcription factors (2 marks). These are required for mediating the reporter response to Wnt signaling. Removal of these sites renders the reporter unresponsive to Wnt signalling (2 marks).

7. a) The posterior segments of primitive arthropods frequently bear appendages. In contrast, the abdominal segments of insects do not develop appendages. What is the molecular basis for this difference in body plan? (2 marks)

Answer: Expression of the distaless gene is required for the growth of arthropod limbs (1 mark). The Ubx gene is expressed in the posterior region of both primitive arthropods (crustacea or velvet worms) and abdomen of insects. However, during insect evolution ubx has acquired the ability to repress distaless expression and therefore the development of limbs in the abdomen (1 mark).

b) What features of the Pax6 protein mean that it is well suited to regulating a wide range of target genes? (2 marks)

Answer: It is able to bind to and regulate a wide range of target genes because it has complex DNA binding characteristics. This results from Pax6 containing two different DNA
binding domains and it is also able to bind to cis-regulatory elements as a heterodimer with other Pax-related transcription factor proteins, as well as binding to DNA as a homodimer.

8. a) Describe two pieces of evidence that a nodal gradient is present in the developing frog embryo. (2 marks)

Answer: Nodal mRNA is expressed in a dorsal to ventral gradient in vegetal hemisphere cells. Phospho-smad2 levels indicate the activity of the nodal signal transduction pathway. There are higher levels of smad2 phosphorylation in the dorsal half of the embryo compared to the ventral side.

b) What are the expression domains of the \textit{brachyury} gene during early vertebrate development? (1 mark)

Answer: Brachyury is expressed in the early migrating mesoderm during gastrulation and in the notochord.

c) Describe the defining features of a phylotypic stage chordate embryo. (3 marks)

Answer: Answer should include reference to all these features: notochord, hollow dorsal nerve tube, branchial arches and pharyngeal slits, repeated segmental muscle blocks (somites) and a tail that extends beyond the posterior opening of the gut (post-anal tail).

9. Provide one example of how external factors such as behaviour or diet can influence the phenotype of an individual via an effect on DNA methylation status. (3 marks)

Various possibilities including Queen Bee vs Worker Bees. Royal Jelly content affecting histone acetylation via HDAC inhibition, gene expression and thereby DNA methylation (1) or Maternal behaviour influencing DNA methylation of the glucocorticoid receptor of the offspring (1) via activation of the serotonin pathway and histone acetylation.

10. Experiments were performed in order to investigate whether the DNA binding protein Cfp1 can distinguish between methylated and unmethylated CpG islands (CGIs). In female mammals, the Xist CGI is methylated on one of the X-chromosomes but is unmethylated on the other. Chromatin-immunoprecipitation was performed on female mouse cells using Cfp1-specific antibodies followed by bisulfite sequencing of the Xist CGI (Cfp1 IP). As a control bisulfite sequencing was also performed on the Xist CGI without prior immunoprecipitation (Input DNA). The filled and open circles represent methylated and unmethylated CpGs, respectively. Uncharacterized CpGs are represented as gaps.
a) Briefly explain what is bisulfite sequencing. (3 marks)
Bisulfite sequencing is a technique to determine the methylation status of individual cytosines (1). The bisulfite treatment converts unmethylated cytosines to uracil whereas methylated cytosines stay as cytosine (1). Hence treatment and comparison of the resulting sequence to that of untreated DNA can show cytosine methylation status (1).

b) Provide an explanation for the pattern of Xist CGI methylation observed for the Cfp1 IP sample compared to the input DNA. (3 marks)
Cfp1 is specific to the unmethylated XIST CGI allele as the IP pulls down predominantly unmethylated cytosines (2). The input is ~50% methylated as one allele should be methylated and one allele unmethylated (1).

c) In females, will the Xist CGI be methylated on the active or inactive X chromosome? Explain your answer. (2 marks)
The active X as XIST should not be expressed on the active chromosome (1). It will be unmethylated on the inactive X as it is expressed and plays a key role in the inactivation process (1).

11. The mammalian polycomb group protein, EZH2, is a histone methyltransferase and is required for repressing the expression of many targets including the MYT1 gene. Analysis of the MYT1 gene shows that DNA methylation normally correlates with the presence of H3K27me3. A series of experiments was performed in order to assess the relationship between EZH2 activity and DNA methylation.

RNA interference was used to knock down expression of EZH2, DNMT1, DNMT3A or DNMT3B in cultured cells and RNA levels of MYT1 and Actin were assessed by RT-PCR (Figure 1). The RNAi control is not expected to affect expression levels of MYT1 or Actin.

Figure 1
a) Provide an explanation for the patterns of MYT1 expression shown in Figure 1. (2 marks) EZH2, DNMT1, DNMT3A and 3B are all involved in repressing MYT1 expression (1) as when they are supressed by RNAi, levels of MYT1 expression increase compared to the control (1).

b) What do you predict will be the effect on MYT1 expression levels if cells are treated with a DNA methylation inhibitor, 5-aza-cytidine? Provide an explanation for your answer. (2 marks)

That MYT1 expression levels will increase (1 mark). The result should be equivalent to performing RNAi on the DNA methyltransferases as shown in Figure 1 (1 mark).

Next Chromatin immunoprecipitation (ChIP) experiments were performed to investigate the association of EZH2, H3K27me3, DNMT1, DNMT3A and DNMT3B with MYT1. These experiments were performed using cells that had undergone RNAi of EZH2 (Fig. 2A) or DNMT1, DNMT3A or DNMT3B (Fig. 2B). Input refers to PCRs performed on chromatin that had not undergone immunoprecipitation. ChIP Ab refers to the antibody used in each chromatim immunoprecipitation.
c) Discuss whether the evidence shown in Figure 2 supports a model for MYT1 in which DNA methyltransferases recruit EZH2 or whether EZH2 recruits DNA methyltransferases. (3 marks)

It does not support a model whereby DNA methyltransferases recruit H3K27me (1) as, when the DNMTs are suppressed by RNAi the presence of H3K27me3 and EZH2 at MYT1 are unaffected (1). Rather it supports the converse as when EZH2 is suppressed the DNMTs are no longer present (1).

12. a) Describe the epigenetic reprogramming events that occur during preimplantation development in mammals and the mechanisms involved. Comment on why this reprogramming is necessary. (7 marks)

Male and female gametes are specialized cells with very different epigenomes (1). Reprogramming is required to return cells to a totipotent state to allow a correct developmental programme to be undertaken (1). It may also be required to reduce the likelihood of trans-generational passing of epigenetic information (1). The paternal genome undergoes a rapid and active demethylation likely involving Tet3 action and production of 5-hydroxymethyl cytosine (1). The maternal genome is also demethylated but predominantly by a passive mechanism (1). The maternal genome is protected from active demethylation by the action of Stella protein (1). The passive mechanism involves preventing the mechanisms that normally maintain DNA methylation and therefore levels are reduced by successive DNA replications and cell divisions (1).

b) Describe how the Beckwith-Wiedemann and Silver-Russel imprinting disorders can arise in humans and explain why they have opposite phenotypes. (5 marks)

These disorders both affect the Igf2-H19 imprinting cluster with Igf2 being involved in growth promotion and expressed from the paternal allele (1). Normally there is DNA methylation of the ICR on the paternal but not maternal alleles that results in mono-allelic paternal
expression of Igf2 (1). In BWS gain of DNA methylation on the maternal ICR results in biallelic Igf2 expression and an over-growth phenotype (1). In SRS loss of paternal ICR methylation results in no Igf2 expression and growth retardation (1). These disorders can also arise by DNA-based mutations within the cluster with the phenotype depending on whether the defective allele is inherited from the father or mother (1).

13. 
   a) Name two tissues that the cardiac neural crest contributes to. (2 Marks)
   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)

   b) Briefly describe some of the evidence for why we think the neural crest contributes to these tissues (2 Marks)
   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)

14.
   A set of primers designed to discriminate between two alleles of an important SNP within a gene are shown below:

   Arrowheads indicate the 3’ end of the DNA. Primers on the top strand are oriented from left (5’) to right (3’). Primers on the bottom strand are oriented from right (5’) to left (3’). The distance in bp between some primers and the site of the SNP is indicated.

   You perform PCR with all of the primers indicated above in a single mastermix on
samples taken from homozygous and heterozygous individuals. Draw bands on the gel below corresponding to the outcome you expect in each case. (6 Marks)

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Answer:

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(2 marks for hom 1, 2 marks for hom 2, 2 marks for the fact both bands will be present in the hets)