BSc Stage 3 Degree Examinations 2018-19

Department:

Biology

Title of Exam:

Advanced Topics in Developmental Biology

Submission deadline:

XXX

Allocation of Marks:

Part A 40 marks

Reference: Daubas et al (2015) Fine tuning the onset of myogenesis by homeobox proteins that interact with the myf5 enhancer. Biology Open 4, 1614-1624

Part B 40 marks


Instructions for Candidates:

- Please read the papers and associated supplementary material
- Answer all questions, from part A and part B, typing on this document, and keeping to the word limit.
- Please do not include a reference list, as the questions should be answerable using the papers assigned and knowledge of the teaching in this module.
- Answer on this document and return to the office.
PART A (40 marks)

Please read the main text and supplementary information of this publication:


Question 1

Describe the rationale for investigating whether the homeodomain transcription factors Meox2 and Msx1 contribute to the regulation of myf5 during the migration of myogenic precursors to the limb.  

(4 marks; 120 words)

Myf5 is expressed in muscle progenitors in the limb, but is not expressed in migrating muscle progenitors (1 mark); activators of myf5 Pax3 and Six1/4 (1/2 mark) are expressed in migrating muscle precursors and act on a well-defined limb enhancer -57/-58kb (1 mark) of myf5; some additional modulators (1 mark) of myf5 expression are expected to define its off (during migration)/on expression (in the limb). Msx1 and meox2 are also expressed (1 mark) in migrating muscle precursors. Previously shown that msx1 represses myoD (1/2 mark), and meox2 mutants have delayed myf5 expression in limb (1/2 mark). (accept sensible combinations of these points up to value of 4 marks)

Question 2

Assess the experimental evidence that Meox2 and Msx1 proteins bind the core element in the myf5 limb enhancer (6 marks). Include and justify whether you think there is stronger evidence for the binding Meox2 or Msx1 to the core element. 

(8 marks; 240 words)

A labelled DNA probe was made from the core enhancer (1 mark) that includes consensus binding sites for homeobox transcription factors (1/2 mark) was used in EMSA/gel shift experiments (1/2 mark) with Meox2 protein was synthesized in vitro. The band shifts show that Meox binds the sequence (1 mark), and it is specific because this binding is competed off with unlabelled core enhancer DNA (1 mark); more data for specificity of the protein nucleic acid interaction is shown by super-shifting the complex with an antibody against Meox2 (1/2 mark). The same experiments are done with an HA-tagged Msx1 and an anti-HA antibody which doesn’t cause a super-shift (1 mark), but causes a loss of the band (1/2 mark). This is possibly due to the Ab interfering with Msx1-DNA interaction (1/2 mark). Mutating Hbx2 (Figure 4) in the core element results in a loss of Meox2 and Mix1 binding/shifting (1 mark). In vivo evidence as well makes case stronger: ChIP-PCR using anti-Meox2 Ab to pull down Meox2-DNA complex in limb region; also done with antiHA on Msx1-HA transgenic mice; shows increase pull down of this DNA region compared to non-limb region (2 marks) (accept sensible combinations of these points up to value of 6 marks)

Using an epitope tagged version instead of antibody to endogenous protein for Msx proteins is less convincing (1/2 mark)/ no super-shift (1/2 mark)/ no cold competitor shown (1 mark) as was done in Meox2 experiment. (accept sensible combinations of these points up to value of 2 marks)
Question 3

Describe the genetic approaches, and explain how they are used, to demonstrate that Msx1 and Meox2 are required in vivo to modulate the expression of myf5 in migratory and limb myogenic precursors. (8 marks; 240 words)

Transgenic embryos expressing nuclear LacZ under the control of the -57/-58 limb enhancer with a Hbox2 mutation results in increased lacZ in the limb; this points to more myf5 expression when this region is mutated; this mutation result in a loss of Msx1 and Meox2 binding in EMSAs; conclude that the element negatively regulates myf5. (2 marks)

Pax3-CRE mice crossed with floxed Msx1 and Msx2 mice results in loss of Msx1/2 in all cells that express Pax3 including migratory muscle progenitor cells.myf5 expression expands in cells delaminating from hypaxial somite; so normally msx prevents myf5 expression in delaminating cells (2 marks)

Meox2 mutant mice crossed with myf5 lacZ reporter mice show decreased limb lacZ, indicating earliest expression of myf5 in the limb requires Meox2 (2 marks)

Transgenic embryos expressing nuclear LacZ under the control of the -57/-58 limb enhancer with all three Hbox sites mutated results in loss of lacZ in the limb, showing these sites are required for myf5 expression in limb; likely through the interaction of meox2 with these sites. (2 marks)

Question 4

Based on this paper and other papers we have covered this term, outline an experimental approach to test whether Shh signalling is required for myf5 expression in the limb muscle precursor cells and whether Shh regulates the activity of the limb enhancer. State your hypothesis, describe your proposed experiments and expected results, and justify your predictions. (10 marks; 300 words)

Sensible programmes of identifying Gli binding sites, mutation of site, reporters, transgenics, use of shh knock out mice and conditional alleles, EMSA...

(Please answer Question 5 based on other papers covered during this module)

Question 5

a) Consider whether the model that proposes two independent lineages of myoblasts (Haldar et al 2008) is consistent with the findings in Conerley et al 2016 and justify your position by referring to and interpreting relevant experimental data. (7 marks; 210 words)
Conerley shows a cell autonomous sequential activity of Myf5 then MyoD driving transcription at the same genetic loci; Haldar shows evidence for (at least) two separate lineages of myoblasts, one myoD driven, one myf5. Good explanations discriminating these ideas.

b) Outline the best evidence that the specification and migration of the slow muscle lineage requires hedgehog signalling in zebrafish.   (3 marks; 90 words)

various evidence accepted for a mark, but needs to say that lack of engrailed and prox1/smyhc in Smu mutants is best evidence for full marks.

PART B
Please read the main text and supplementary information of this publication:


Question 1

a) Describe and critically assess the data that HtrA1 expression is regulated by members of the FGF family.   (6 marks; 180 words)

Answer:

- There is overlap between FGF expression and known areas of FGF activity, as shown by dp-Erk staining, in the blastopore/marginal zone at the gastrula stage (1 mark).

- HtrA1 is also expressed in the anterior of the neural plate, however, this does not match that of FGF8 very well, as HtrA1 expression is more extensive than that of FGF8 (1 mark).

- FGF4 and FGF8 both induce the expression of HtrA1 in animal cap explants which indicates that FGF signalling is sufficient for HtrA1 expression (1 mark).

- Despite the overlap of expression and induction of expression by FGF in animal cap explants the evidence indicates FGF signalling is not required in vivo for HtrA1 expression. Figure s3 shows that dnFGFR1 and dnFGFR4 both block the expression of brachyury (a known target of FGF regulation) but expression of HtrA1 is unaffected. Critical (2 marks)
The tone of the Answer is important because it must involve some caution as to the overall conclusion. The data do suggest a relationship but it is far from conclusive and questions remain. Should conclude that there are probably other signals involved in regulating HtrA1 expression (1 mark)

b) Is there any evidence to suggest that HtrA1 has a role in regulating FGF signalling before the onset of zygotic transcription? Explain your reasoning.

(2 marks; 60 words)

**Answer:** There is no evidence to support a role for HtrA1 in regulating FGF signalling before zygotic transcription. The rt-PCR analysis in Figure S2 indicates that HtrA1 mRNA is not detected until early gastrula stage 10. This means that there is no maternally deposited mRNA and HtrA1 is only expressed after the onset of transcription at the mid-blastula transition.

c) With reference to relevant experimental evidence in this paper, and other papers that we have covered this term, propose a mechanism to explain the observation that injection of HtrA1 mRNA induces ectopic expression of the FGF4 gene.

(3 marks; 90 words)

**Answer:** The likely mechanism involves the known autocatalytic loop that exists between FGF4 and brachyury. Evidence presented in this paper indicates that HtrA1 promotes and extends the range of FGF signalling in the embryo. This also results in an expanded domain of brachyury expression in the embryo. HtrA1 also induces brachyury expression in animal cap assays (2 marks). Previous studies have shown that brachyury is able to activate the expression FGF4, therefore HtrA1 activation of FGF signalling will likely lead to increased/ectopic FGF4 expression (1 mark).

**Question 2**

a) Describe the evidence that the biological effects of HtrA1 in the embryo require extracellular FGF signalling.

(3 marks; 90 words)

**Answer:**

- Injection of dnFGFR1 mRNA blocks endogenous FGF signalling and gives rise to a phenotype of enlarged anterior and loss of posterior structures. Conversely, HtrA1 overexpression results in reduced head and ectopic posterior (tail) structures. co-injection of dnFGFR1 rescues head loss and ectopic tail formation induced by HtrA1.
- dnFGFR1 also rescues/blocks ectopic brachyury expression induced by HtrA1 and dnFGFR4 inhibits the ectopic formation of neurons induced by HtrA1.
- Both dnFGFR1 and dnFGFR4 function by blocking extracellular FGF signalling, therefore extracellular FGF signals are required for the activity of HtrA1.

b) Describe how it was determined which functional domain of the HtrA1 protein is required for its activity in early development.

(2 marks; 60 words)

**Answer:** It was determined that the trypsin-like catalytic domain of HtrA1 is required for its activity by generation of the HtrA1-delta-trypsin deletion mutant and the HtrA1 [S307A] mutant in which the catalytic serine 307 is replaced with alanine. It was shown that, unlike wild-type HtrA1,
overexpression of these mutant proteins does not induce the formation of tail-like structures and inhibit anterior development.

c) Describe an experimental design to investigate whether the HtrA1 protein is secreted from Xenopus cells.  

Answer: In order to gain full marks for this question an approach that involves direct detection of the protein in the extracellular environment is required. I don’t think using a proxy assay of biological activity deserves full marks here.

There are a number of possible approaches and any well-described, plausible design will attract full marks. The most obvious one covered by lecture material would involve injection of synthetic HtrA1 into oocytes, culturing for a period and then detecting the presence of the protein in the oocyte culture medium (2 marks). The detection of the protein could be done by western blot using the antibody used in Figure 2R (1 mark). Other approaches might involve injection of the synthetic mRNA into embryos allow them to develop and detecting the presence of secreted protein in the blastocoel fluid. Other approaches might involve the detection of a secreted tagged protein. Lots of workable approaches are possible.

Question 3

a) Describe and critically assess the evidence that the neutralising HtrA1 antibody used in this paper inhibits HtrA1 activity. Suggest how this might be further investigated.  

Answer: The Answer needs to acknowledge that there is actually little evidence provided that the neutralising antibody inhibits HtrA1 function. The evidence is purely correlative. (1 mark)

Figure S5 shows that injection of the antibody, but not the pre-immune serum, leads inhibition of brachyury, n-tubulin and sizzled expression, and expansion of anterior neural markers similar to that caused by inhibition of HtrA1 with antisense morpholinos. The similar effects are suggestive that the antibody might also inhibit HtrA1. (2 marks)

Some sort of functional assay is required. A number of possibilities here. I think the most obvious assays are the biglycan and glypican assays in Figure 7. it is shown that the MO blocks biglycan and glypican degradation by endogenous HtrA1. This could be adapted to see if blastocoel injection of the antibody also blocks degradation. Other well described and plausible alternatives will attract marks. (2 marks)

b) With reference to the experimental evidence, discuss whether the effects of manipulating HtrA1 activity in development and the data in Lamb and Harland (1995) both support a role for FGF signalling in anteroposterior patterning.  

Answer: The Answer needs a clear statement on whether the candidate considers the two studies to be in agreement. the appropriate response would be a broad yes. On the basis that HtrA1 works by modulating FGF signalling there are plenty of data in this paper to support the role of FGF as a posteriorising factor (1 mark).
Lamb and Harland study indicates that in a gastrula stage animal cap assay FGF induces posterior neural tissue and is able to posteriorises neural tissue induced by the BMP inhibitor noggin. Noggin induces neural tissue expressing anterior markers like Otx2. The addition of FGF induces posterior markers like Hoxb9, as well as intermediate(hindbrain) markers like Krox20 (2 marks).

Posteriorising effects of FGF overexpression, including loss of head structures and ectopic tails. In keeping with this HtrA1 overexpression inhibits anterior marker gene expression in whole embryos and neural plate explants. Conversely, HtrA1 overexpression posteriorises gene expression in neural plate explants. Might mention that the in situ data for markers like Hoxb9 are lacking here (2 marks).

Data from HtrA1 knockdown are also in keeping with a role for FGF in in posterior development. Posterior truncations in HtrA1 knockdowns are similar to those resulting from FGF inhibition.

Question 4

a) Compare and contrast the mechanism of HtrA1 function with that of other feedback regulators of FGF signalling discussed in this paper and during this term’s lectures. (3 marks; 90 words)

Answer: An important take home from this Answer should be that HtrA1 is secreted and acts non-autonomously. This contrasts with other feedback regulators of FGF signalling discussed in the paper and in the lectures, which all act cell autonomously (1 mark). There are lots of examples, that could be used to illustrate this point, some are mentioned in the text, however, the best examples are probably brachyury(positive regulator), which acts via transcriptional regulation of FGF4 and DUSP5 (negative regulator) which acts to inhibit FGF signal transduction via dephosphorylating MAPK erk. Other examples if accurately discussed will also attract marks (2 marks).

b) Describe and critically assess the evidence that HtrA1 regulates the stability of syndecan, biglycan and glypican in the developing embryo. (4 marks; 120 words)

Answer: The tone of the Answer should indicate that these data are bit of a mixed bag and somewhat incomplete. It might also be said that there are caveats on the interpretation because the experiments are done using epitope tagged proteoglycans rather looking at the endogenous proteins (1 mark).

The best data are for biglycan where it is shown that HtrA1 overexpression cause degradation of biglycan and MO mediated HtrA1 inhibition leads to elevated biglycan levels. The data with glypican is less convincing. HtrA1 knockdown leads to increased glypican levels but HtrA1 knockdown does not affect glypican levels. Similarly with syndecan it is shown that overexpression causes degradation of syndecan but there are no data relating to HtrA1 inhibition (3 marks)
c) Discuss the evidence that HtrA1 function is not restricted to roles in regulating FGF signalling in mesoderm induction and neural patterning in early development.

(2 marks; 60 words)

Answer: There are two possible approaches to Answering the question and either is perfectly valid and will attract marks if accurately described. Both Answers can be obtained by careful reading of the paper introduction and discussion.

**Approach 1:** HtrA1 functions by modulating other signalling pathways. HtrA1 binds to and inactivates members of the TGF-beta family (bmp2 and bmp4 in cultured cells) and modulates IGF signalling by cleaving IGFBP5.

**Approach 2:** HtrA1 regulates other FGF related-processes e.g. bone development (ossification and remodelling). Association with FGF angiogenic function in age-related macular degeneration.