Module Code: BIO00071H

Examination Number:_______________

BSc Degree Examinations 2019-0

Department: BIOLOGY

Title of Exam:
Advanced Topics in Molecular Biology

Time Allowed:
24 Hours

Time Recommended:
2 hours 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:
Answer all questions within the line limits stated on the examination paper. Please use Arial font, size 11 or larger.
Content beyond the line limit will not be marked.
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).
Part A: 40 marks

Questions based on Gokhale et al. (2020). Altered m\(^6\)A modification of specific cellular transcripts affects Flaviviridae infection. Molecular Cell 77, 542-555

1. a) Gokhale et al. studied the effect of DENV, ZIKV, WNV, and HCV infection on the m\(^6\)A epitranscriptome. Why was it advantageous to study multiple viruses rather than focus on a single virus? (2 marks, 6 lines)

   b) Changes in m\(^6\)A modification of host transcripts during infection were determined using MeRIP-seq. Why were both the input and immunoprecipitated fractions sequenced? (2 marks, 6 lines)

   c) Summarize the evidence that the authors are using when they conclude that Flaviviridae infection alters m\(^6\)A modification of specific transcripts. (5 marks, 10 lines)

2. Flaviviridae infection triggers an increase in m\(^6\)A on RIOK3 transcripts and a decrease on CIRBP transcripts. Evaluate the evidence that these changes are triggered via different cellular pathways. (5 marks, 15 lines)

3. RIOK3 protein expression increased following infection. The authors state that they found no significant change in nuclear export or mRNA stability of RIOK3 mRNA during infection. Briefly describe how nuclear export and mRNA stability can be assessed experimentally. (4 marks, 8 lines)

4. Evaluate the evidence that the authors are using to conclude that RIOK3 translation was increased by infection. (3 marks, 8 lines)

5. Explain the design of the luciferase reporter assay as shown in Figure 3 and the relevance for this study. (6 marks, 12 lines)

6. Evaluate the evidence that m\(^6\)A modification influences alternative splicing of the CIRBP transcript and provide a mechanistic explanation. (7 marks, 12 lines)
7. In the discussion (pg. 550) the authors describe possible mechanisms that could be responsible for modulating the selective m^6A modification during infection. Briefly describe a follow up experiment that would allow you to further investigate one of these mechanisms. (6 marks, 12 lines)

Part B: 60 marks.

8. Phase separation analysis based upon observation of droplet formation in vitro was performed for the low complexity region of Protein A, either in its unphosphorylated (Protein-A\textsubscript{LCR}) or phosphorylated form (Phospho-Protein-A\textsubscript{LCR}). Droplet formation was assessed in the presence of different concentrations of RNA and using a range of Protein A concentrations.

![Graph showing droplet formation]

Summarise how phosphorylation and presence of RNA influences phase separation of Protein A. Suggest a mechanistic explanation for the data. (5 marks, 12 lines)

9. a) The chromatin reader protein ARD2 and the mediator subunit MED1 are both mammalian proteins predicted to have C-terminal intrinsically disordered regions. Describe a series of experiments that would allow you to investigate whether ARD2 and MED1 are able to form biomolecular condensates in vivo, and to determine whether they co-localize. (6 marks, 10 lines)
b) You find that ARD2 and MED1 co-localize at super-enhancers. Describe how you could test whether the ARD2-MED1 condensates are sites of active transcription.  
(3 marks, 8 lines)

c) How do the structures of super-enhancers help promote the formation of biomolecular condensates?  
(2 marks, 4 lines)

10. Protein IFX2 is a component of a membraneless organelle. The dynamics of fluorescently-labelled IFX2 were analyzed by Fluorescence Recovery after Photobleaching (FRAP). Bleaching was carried out on the membraneless organelle and it was observed that recovery did not fully restore pre-bleached fluorescence levels (1st FRAP curve below). The same organelle was then subjected to a 2nd FRAP treatment and this time recovery did restore pre-bleached (post 1st FRAP) levels. The dashed line represents the pre-bleached fluorescence levels.

![Fluorescence Recovery Graph](image)

Provide an explanation for the data shown in the graph.  
(4 marks, 8 lines)
11. Outline, with an example, one possible mechanism by which antisense transcription controls gene expression. (3 marks, 5 lines)

12. Scientists are investigating whether a locus is imprinted. They believe the gene encoding Protein X is expressed by the maternally derived chromosome and repressed on the paternally derived chromosome by antisense transcription of IncRNA Y.

a) Outline a method that could be used to determine whether their hypothesis is correct. (5 marks, 10 lines)

b) What controls should the scientists include in the experiment? (2 marks, 4 lines)

c) Protein X is involved in repressing growth. The scientists identified a frameshift mutation close to the beginning of the gene encoding Protein X. What phenotypes might you expect if this mutation is maternally inherited compared with paternally inherited? (2 marks, 4 lines)

13. A group of scientists investigated which region of the Xist IncRNA is required for recruitment of the polycomb repressive complexes. They generated XY embryonic stem cells expressing a series of transgenes encoding the full-length and mutant Xist transcripts shown below. They induced expression of the transgene and quantified what percentage of Xist domains co-localised with enrichment for H3K27me3 and H2AK119Ub1 (shown in the graph below).
a) Suggest why the scientists used XY embryonic stem cells for this experiment. (3 marks, 6 lines)

b) What conclusions can be made from the data regarding polycomb repressive complex recruitment? Comment on whether these data are consistent with current models in the literature. (5 marks, 10 lines)

14. a) You are a kinetoplastid geneticist investigating how amastigote-stage virulence factors in *Leishmania* are regulated. On what level would you expect these to be regulated and why? (4 marks, 6 lines)

b) You send off mRNA derived from *Leishmania*-infected macrophages (72hr post infection) for HiSeq. Describe how you could distinguish the parasite mRNAs from the host macrophage and explain how this distinction arises. (3 marks, 5 lines)
c) What parasite lifecycle stage would you expect all your isolated mRNAs to be from?  
(1 mark, 2 lines)

d) What distinguishes the transcription of mRNAs from human-infective *Leishmania* from the VSG surface antigens of human-infective *T. brucei*?  
(1 mark, 2 lines)

e) How would you determine whether your parasite-derived mRNAs are specific to *Leishmania* amastigotes?  
(2 marks, 3 lines)

f) Describe an experiment to isolate interacting *trans*-regulators which bind amastigote mRNAs.  
(4 marks, 6 lines)

g) Proteomic data has just been published for amastigote-stage *Leishmania* parasites. You find some interesting candidate proteins. How would you test their relevance to parasite virulence?  
(1 mark, 3 lines)

h) Interestingly, the mRNA levels of many amastigote-specific proteins are constitutively expressed in all lifecycle stages. Provide a mechanistic explanation for this observation.  
(4 marks, 6 lines)

End of Exam