UNIVERSITY OF YORK
BSc Stage 3 Degree Examinations 2017-18

Department: BIOLOGY

Title of Exam: Transcription and Cancer

Time allowed: 2 hours
Total marks available for this paper: 100

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

For marker use only:

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Total as %

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SECTION A: Short Answer / Problem / Experimental Design questions

Answer all questions in the spaces provided

Mark total for this section: 100

1. RNA polymerases (pols) I, II and III evolved from a shared progenitor.

   a) Which components of the pol II preinitiation complex have clear homologues in the preinitiation complexes of pols I and III? (4 marks)
   
   TFIIB, TFIIE, TFIIF and several subunits of the pols themselves.

   Very few remembered the homology between the RNA polymerase subunits. Many included TBP, which is present in all 3 PICs; I only gave ½ mark for this, as it is a shared polypeptide rather than a homologue.

   b) Which RNA polymerase subunit is recurrently mutated in meningiomas? (2 marks)
   
   POLR2A (1 mark), the largest subunit of pol II (1 mark).

   Most remembered this.

   c) What evidence implicates pol III in organismal aging? (6 marks)

   Lifespan can be extended in yeast, worms & fly model organisms (2 marks) by decreasing expression of pol III-specific subunits (1 mark) using RNAi (1 mark), or genetic mutation (1 mark) or tagging with an inducible degron (1 mark). Other details can score marks.

   Considerable variation in the level of detail provided. Many mentioned gut deterioration and/or TOR/rapamycin/Maf1, which are relevant.

   LO1: Explain how transcription complexes assemble and function at distinct types of gene.
   LO4: Explain the importance of transcription factors for cancer.

2. ENL is a subunit of the Super Elongation Complex (SEC). ChIP-Seq was used to identify protein-coding genes where ENL is detected, genome-wide. The image below compares pol II occupancy, relative to transcription start sites,
between ENL target genes and genes where ENL was undetected. It also shows the effect of ENL depletion on pol II occupancy.

![Graph showing ENL target genes, mock depletion and ENL target genes, ENL depletion.](image)

a) What conclusions can you draw from these data about the role of ENL? (3 marks)

ENL stimulates pol II occupancy (2 marks), especially in the transcribed region (1 mark).

High success rate.

b) Why is a peak of pol II observed at the start of many genes? (2 marks)

Promoter escape is rate limiting for many pol II-transcribed genes.

High success rate.

c) How would you expect the above image to differ if the pol II antibody used for ChIP-Seq had been specific for the C-terminal domain of POLR2A when it is phosphorylated on Ser5? Explain your answer (2 marks).

Signal would be strongest near the start & decrease gradually towards the end of the gene (1 mark), as Ser5 is phosphorylated by TFIIH at the promoter & then gradually dephosphorylated as pol II progresses through the coding region (1 mark).

High success rate.

d) ENL contains a YEATS domain that binds acetylated histone H3. How would you test if a substitution in this domain disrupts binding to the acetylated tail of histone H3 in vitro? (2 marks)
Peptide pull-down assay using WT or mutant ENL protein. Other answers could score marks here (e.g. isothermal calorimetry).

Quite variable.

e) How would you test if the same substitution disrupts binding of ENL to the MYC gene in vivo? (5 marks)

Transfect cells with tagged versions of WT or mutant ENL (2 marks). Carry out ChIP-PCR with antibody against the TAG & primers to amplify MYC (2 marks). Western blot to confirm equal expression of WT & mutant ENL (1 mark). Alternatively, a tag may not be necessary if the endogenous ENL is depleted before transfection.

Most got ChIP-PCR, but few remembered to consider the issues of distinguishing mutant from WT and comparing their levels of expression, which is crucial in making deductions about the effects of a mutation. A few thought that “in vivo” meant in an animal model, which would be much more challenging, but the term is commonly used to include living cells in culture.

f) In some leukaemias, the ENL and MLL1 genes are fused together by chromosomal translocations. How does this drive leukaemogenesis? (2 marks)

The MLL1 domain of the fusion recruits SEC and DOT1L to inappropriate targets via the ENL domain (1 mark), thereby aberrantly activating genes that promote proliferation and inhibit differentiation, e.g. HOX genes (1 mark).

Most got this at least partly right.

LO1: Explain how transcription complexes assemble and function at distinct types of gene.
LO2: Discuss the impact of chromatin on gene transcription.
LO3: Explain how transcription is regulated.
LO4: Explain the importance of transcription factors for cancer.
LO6: Interpret data from experiments investigating transcription.

3. GSK343 is a drug that was developed to inhibit EZH2. When bladder cancer cells were treated with GSK343, expression of the IGFBP3 gene increased.
a) How would you test if this response to GSK343 is due to inhibition of EZH2, rather than an off-target effect? (2 marks)

Test if siRNAs that knockdown EZH2 expression can also induce IGFBP3 expression (2 marks). Or test if GSK343 induces IGFBP3 in cells where EZH2 has been depleted or deleted. Other answers could score marks here.

High success rate.

b) If GSK343 releases EZH2 from the IGFBP3 gene, how would you expect this to affect transcription complex assembly and function? (6 marks)

EZH2 recruits DNA methyltransferases and histone deacetylases that repress transcription complex assembly. Its inhibition is expected to increase transcription complex assembly and function in several ways. SWI/SNF recruitment is likely to increase, as this is antagonised by EZH2, resulting in movement and eviction of nucleosomes (1/2 mark). Histone acetylation is likely to increase (1/2 mark), facilitating recruitment of TFIID (through TAF1 recognition of H4Ac) (1/2 mark) and Brd4 (1/2 mark). Brd4 can evict nucleosomes (1/2 mark) and recruit P-TEFb, that increases promoter clearance (1/2 mark). DNA methylation at CpG is likely to decrease (1/2 mark), allowing recruitment of MLL1/2, which trimethylate H3K4 (1/2 mark), to allow recruitment of the chromatin remodeler CHD1, that shifts nucleosomes (1/2 mark), and also aid TFIID recruitment through TAF3 (1/2 mark). Increased assembly of TFIID is expected to stimulate assembly of TFIIA, TFIIIB, pol II + TFIIE and TFIIH, i.e. the complete pol II preinitiation complex (1 mark).

Most predicted correctly that GSK343 would increase transcription complex assembly and function. Varying levels of detail were provided & marks were awarded accordingly. Other points also scored marks, if relevant and accurate.

c) Treatment with GSK343 decreased the proliferation of the bladder cancer cells relative to matched cells treated with vehicle (Ctrl). As shown below, this response was maintained if the cells were transfected with a non-targeting siRNA (si-Ctrl) but was abolished by transfection with siRNA that depleted IGFBP3 (si-IGFBP3). How would you interpret this? (2 marks)
We have been told that GSK343 suppresses proliferation and induces IGFBP3 expression. As suppression of proliferation is lost when IGFBP3 is depleted, the data suggest that suppression of proliferation by GSK343 requires induction of IGFBP3 (2 marks).

Highly variable success rate. Many focused on the relatively small increase in proliferation between controls with si-Ctrl and si-IGFBP3, rather than the much bigger difference in the response to GSK343.

d) How does EZH2 promote cancer development? (2 marks)

It silences the p16INK4a and p15INK4b tumour suppressor genes (1 mark). This allows inappropriate phosphorylation of RB, removing a barrier to growth and cell cycle progression (1 mark).

Some went into more detail concerning the mechanism of repression of tumour suppressor genes (recruitment of HDACs & DNMTs etc); this was fine.

e) What observation raises concern about the use of EZH2 inhibitors as a therapeutic strategy? (2 marks)

Inactivating mutations in EZH2 are recurrently found in certain malignancies (e.g. myelodysplastic syndrome (1 mark) suggesting that EZH2 may have tumour suppressive function in some contexts (1 mark).
Some remembered this, but most did not.

 LO1: Explain how transcription complexes assemble and function at distinct types of gene.
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 LO4: Explain the importance of transcription factors for cancer.
 LO5: Discuss how transcription is being targeted as a way to tackle cancer.
 LO6: Interpret data from experiments investigating transcription.

4. a) By what mechanisms does MDM2 neutralise tumour suppressor activity? (4 marks)

MDM2 binds the N-terminal transactivation domain of p53, so it cannot recruit TFIID (1 mark). It can sequester p53 in the cytoplasm (1 mark) or polyubiquitinate it for proteasomal degradation (1 mark). MDM2 also binds & neutralizes RB (1 mark).

High success rate.

b) What viral oncoproteins mimic these effects of MDM2? (2 marks)

HPV E6 directs polyubiquitination of p53 (1 mark) and HPV E7 binds the pocket of RB (1 mark). Other answers could score marks here, such as SV40 large T or adenoviral E1A and E1B.

Most remembered HPV E6 & E7.

c) Nutlins are drugs that were developed to protect p53 from MDM2. How do they function? (1 mark)

Nutlins occupy the p53-binding pocket of MDM2 to block interaction.

High success rate.
d) CX5461 is an inhibitor of transcription by RNA polymerase I that is currently in clinical trials. It was shown to release p53 from MDM2 in lymphoma cells. How does it produce this effect? (2 marks)

Inhibition of rRNA synthesis by pol I causes accumulation of free ribosomal proteins (1 mark), which bind to MDM2 and block its interaction with p53 (1 mark).

High success rate.

e) Describe, with controls, how you would design an experiment to test if CX5461 can also release p53 from MDM2 in breast cancer cells. (5 marks)

Immunoprecipitate MDM2 from cells +/- CX5461 and blot for p53 (2 marks). Blot for MDM2 to confirm that the IP has worked (1 mark). The reciprocal is equally good (i.e. IP p53 and blot for MDM2). Include a negative control IP with preimmune serum or similar (1 mark). A proteasomal inhibitor might facilitate detection of the interaction, as p53 can be degraded after binding MDM2 (1 mark). Other answers could score marks here.

Most got this right, although not many thought of including a proteasome inhibitor to increase the stability of the MDM2/p53 complex. It could be difficult to detect the interaction if p53 is rapidly degraded after binding MDM2.

LO3: Explain how transcription is regulated.
LO4: Explain the importance of transcription factors for cancer.
LO5: Discuss how transcription is being targeted as a way to tackle cancer.

5. The image below shows western blots for p53, p21, p16INK4a and RB, in lysates from cells transfected with an empty vector (lane 1) or a vector encoding an oncogenic Ras mutant (lane 2).
a) What important control is missing from the above? (2 marks)

A loading control is needed to demonstrate equal amounts of total protein in lanes 1 and 2, e.g. blot for actin or GAPDH.

High success rate.

b) Briefly explain how an oncogenic Ras mutant can trigger the increases in p53 and p21 proteins that are suggested by the western blots. (5 marks)

Oncogenic Ras induces expression of Arf (1 mark), which inhibits MDM2 (1 mark) thereby stabilising p53 (1 mark). P53 activates transcription of the p21 gene (1 mark) by recruiting TFIID & p300 (1 mark).

Highly variable success rate here, ranging from 0 to 5 marks.

c) Explain why RB migrates further in lane 2 than in lane 1. (3 marks)

Oncogenic Ras also induces expression of p16/INK4a (1 mark), which inhibits cyclin D-dependent kinases (1 mark) leading to the dephosphorylation of Rb and hence its increased mobility by SDS-PAGE (1 mark).

Most identified that the mobility shift reflects a change in phosphorylation of RB, but not many traced this to the induction of p16. Quite a few thought that erroneously that phosphorylation increases mobility in SDS-PAGE, rather than decreasing it.

d) How would you test if the oncogenic Ras has induced senescence in these cells? (1 mark)
Stain for senescence-associated beta-galactosidase.

High success rate.

e) Senescence is generally a mechanism of tumour-suppression, but recent data suggest that it can be associated with enhanced risk of malignancy. How can senescence promote malignancy? (2 marks)

Tumour cells can escape from senescence & show activation of the Wnt signalling pathway that enhances their malignancy (1 mark). The senescence-associated secretory phenotype (SASP) can promote epithelial to mesenchymal transition (EMT) (1 mark).

High success rate.

f) The data below summarize changes in expression of the RB (RB1), p53 (TP53) and SOX2 genes when cells were transfected with a non-targeting control shRNA (shNT), shRNA targeting p53 (shTP53), shRNA targeting Rb (shRB1), or shRNAs targeting p53 and Rb (shTP53/RB1). What conclusions can you draw about the control of SOX2 expression by p53 and Rb? (3 marks)
SOX2 is repressed by Rb (1 mark) and p53 (1 mark) in a redundant manner (1 mark).

Most got that RB & p53 repress SOX2, but fewer made the point that this control is redundant. In retrospect, I should have given 2 marks for mentioning redundancy and only ½ mark each for identifying that SOX2 is repressed by RB & p53.

LO3: Explain how transcription is regulated.
LO4: Explain the importance of transcription factors for cancer.
LO6: Interpret data from experiments investigating transcription.

6. The BET inhibitor JQ1 suppresses proliferation of the SUM59 breast cancer cell line. However, a resistant clone (SUM59R) arose from the parental SUM59 cells. To identify molecular changes that might explain this resistance, ChIP-seq analysis was carried out using antibodies against BRD4 and acetylated lysine 27 of histone H3 (H3K27ac), with the parental and resistant cell lines treated with JQ1 or DMSO (control). Differences were seen between parental and resistant cells at the SOD2 gene, as shown below.
a) How would you interpret these data? (4 marks)

BRD4 binding coincides with H3K27 acetylation (1 mark) at the promoter of the SOD2 gene and 2 regions upstream (1 mark). JQ1 suppresses BRD4 binding (1 mark) in the parental but not the resistant cells (1 mark).

Most scored 2-3 marks here. Hardly anybody mentioned the fact that the BRD4 and H3K27ac peaks coincide; perhaps that seemed too obvious to mention.

b) Based on the data above, suggest a mechanism that might account for the resistance of SUM59R cells to JQ1 (3 marks)

Several answers could score here, such as increased drug efflux or mutation of BRD4 to reduce its affinity for JQ1.

High success rate.

c) Although the Wnt pathway appeared normal in SUM59R cells, it has been implicated in other cases of resistance to BET inhibitors. How does activation of the Wnt pathway bypass BRD4 inhibition? (2 marks)

The Wnt pathway activates key targets of BRD4 such as MYC.

A lot of people simply described how Wnt signalling can activate transcription, without making the important that it can induce key targets of BRD4.

d) How is the Wnt pathway activated in most cases of colorectal cancer? (2 marks)

Mutation of the APC tumour suppressor.
A lot of people described how Wnt signalling can be stimulated by inflammatory fibroblasts in the microenvironment; I gave ½ mark for this, although it is not responsible for the initial activation of the pathway.

LO2: Discuss the impact of chromatin on gene transcription.
LO3: Explain how transcription is regulated.
LO4: Explain the importance of transcription factors for cancer.
LO5: Discuss how transcription is being targeted as a way to tackle cancer.
LO6: Interpret data from experiments investigating transcription.

7. Acute promyelocytic leukaemia (APL) is usually driven by a specific chromosomal translocation that fuses the PML and RARalpha genes.

a) How does this fusion drive leukaemia? (2 marks)

The PML-RAR fusion binds and inhibits genes required for differentiation (1 mark). It also disrupts PML nuclear bodies that regulate senescence through p53 (1 mark).

Fairly high success rate.

b) Why does arsenic trioxide treatment deplete PML-RAR fusion proteins but not wild-type RAR? (5 marks)

Arsenic trioxide binds and oxidizes Cys residues in PML (1 mark) to generate intermolecular disulphide bonds responsible for multimerization (1 mark). The multimers attract a SUMO ligase and become polySUMOylated (1 mark). The polySUMO chains recruit a ubiquitin ligase, leading to polyubiquitination (1 mark) and proteasomal degradation (1 mark).

High success rate.

c) Propose an experiment to test if removal of the PML-RAR fusion involves its ubiquitination. (4 marks)
A variety of answers could score marks here. For example, western blotting for PML-RAR in +/- a proteasome inhibitor; immunoprecipitation of PML-RAR from cells treated with or without As2O3, followed by blotting the precipitate for ubiquitin. Or one could deplete ubiquitin or ubiquitin ligases by RNAi and blot to see if this reduces the effect of As2O3 on PML-RAR.

Most scored well here.

d) What experimental technique would you use to test if arsenic trioxide affects expression of the mRNA that encodes PML-RAR?  

Northern blot or RT-PCR.

High success rate. I burst into tears whenever somebody got this wrong.

e) The therapeutic efficacy of arsenic trioxide is enhanced by all-trans retinoic acid. What is its mechanism of action?  

ATRA triggers a conformational change in the ligand-binding domain of the RAR part of the PML-RAR fusion (1 mark) that releases corepressors and recruits coactivators (1 mark), thereby stimulating transcription of differentiation genes (1 mark).

High success rate.

f) How does the treatment of APL with all-trans retinoic acid resemble the treatment of breast cancer with tamoxifen?  

Tamoxifen triggers a conformational change in the ligand-binding domain of oestrogen receptor (1 mark) to control the binding of cofactors and transcription of target genes (1 mark).

High success rate.