AB8D The Control of Gene Expression: Depth and Exam Drill

Total Marks

AQA 7402 · Calculator allowed · about 150 minutes

Name: _____ Date: ____ / ____ / _____

Answer ALL questions. Show all your working.

1 This question tests definitions of core regulatory DNA elements involved in prokaryotic gene control.

(a) State the role of a promoter in transcription. **(1)**

(b) State what an operator is in the context of a prokaryotic operon. **(1)**

(Total for Question 1 is 2 marks)

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2 The lac operon is a classic example used to explain inducible control of gene expression in prokaryotes.

Explain how the lac operon allows E. coli to produce enzymes for lactose metabolism only when lactose is present. In your answer include the roles of the repressor protein, the operator, and allolactose. You should make the biological mechanism clear and concise.

(Total for Question 2 is 6 marks)

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3 This question concerns mRNA stability and steady state abundance. A gene X produces mRNA that is transcribed at a constant rate R transcripts per minute and degraded with first order kinetics with half-life t1/2. The degradation rate constant k = ln 2 / t1/2.

(a) Derive an expression for the steady state number of mRNA molecules Nss in terms of R and k. **(2)**

(b) If R = 120 transcripts per minute and t1/2 = 5.0 minutes, calculate Nss. Give your answer to 3 significant figures. **(3)**

(c) If a drug reduces the mRNA half-life to 2.0 minutes but leaves R unchanged, calculate the new Nss and state the fold change in steady state mRNA abundance (new/old). Give answers to 3 significant figures. **(3)**

(Total for Question 3 is 8 marks)

4 A researcher measures binding affinity of a transcription factor TF1 to a promoter fragment using an electrophoretic mobility shift assay (EMSA). They incubate increasing concentrations of TF1 with a fixed amount of labelled DNA probe and measure fraction bound. Data are shown below.

(a) Estimate the dissociation constant Kd for TF1 from the data by identifying the TF1 concentration at which fraction bound is about 0.5. Give your estimated Kd in nM and justify your choice from the table. **(3)**

(b) Using the model fraction bound = [TF]/(Kd + [TF]) and Kd = 6.5 nM, calculate the theoretical fraction bound at 2 nM and compare with the measured value. Give the calculated fraction bound to 2 decimal places and state the percent error relative to the measured value. **(3)**

(c) Suggest two experimental reasons why the measured fraction bound at low concentrations might be lower than the simple equilibrium model predicts. **(3)**

(Total for Question 4 is 9 marks)

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5 Polymerase chain reaction (PCR) is used to amplify a 1.2 kb fragment. Assume perfect doubling each cycle, but primer-dimer and inefficiencies reduce per-cycle efficiency to 90% effective doubling (i.e. multiply by 1.9 each cycle).

(a) Starting from a single copy of the target, write an expression for the number of copies after n cycles with 90% efficiency per cycle. **(2)**

(b) Calculate the number of copies after 30 cycles. Give your answer in scientific notation to 3 significant figures. **(4)**

(Total for Question 5 is 6 marks)

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6 DNA methylation of a promoter region is measured before and after treatment with a drug. At baseline methylation fraction averaged over the promoter is 0.62 with standard error 0.03 (n = 6). After treatment average methylation fraction is 0.41 with standard error 0.02 (n = 6).

(a) Calculate the percent decrease in mean methylation after treatment (use (baseline - treated)/baseline x 100). Give your answer to 2 significant figures. **(2)**

(b) Using the standard errors given, calculate the standard error of the difference in means assuming independent samples (SEdiff = $\sqrt{SE1^2 + SE2^2}$). Give your answer to 2 significant figures. **(2)**

(c) State whether the change in methylation is likely to be statistically significant at approximately the 95% level by comparing the difference in means to twice the SEdiff. Show your comparison and conclusion. **(2)**

(Total for Question 6 is 6 marks)

7 Mutations in coding sequences can alter protein products.

(a) Define a missense mutation and a nonsense mutation in coding DNA. One sentence each. **(2)**

(b) A coding sequence has 900 nucleotides. A single base substitution introduces a stop codon at nucleotide position 601 (counting from the first base of the start codon). How many amino acids long is the truncated protein? Show working. **(2)**

(Total for Question 7 is 4 marks)

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8 Compare repressible and inducible operons in prokaryotes.

(Total for Question 8 is 4 marks)

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9 A western blot experiment measures protein A expression relative to a housekeeping protein H. Densitometry yields integrated intensity for protein A and H for three biological replicates in control and treated cells as below.

(a) Calculate normalised A/H for each replicate and the mean normalised expression for control. Give mean to 3 significant figures. **(3)**

(b) Calculate mean normalised expression for treated samples to 3 significant figures. **(2)**

(c) Calculate the percent expression in treated relative to control (treated/control x100) to 2 significant figures. **(1)**

(Total for Question 9 is 6 marks)

10 Synoptic scenario. A signalling pathway in hepatocytes regulates expression of enzyme CYPZ which metabolises a drug. A membrane receptor activation leads to phosphorylation cascade, nuclear translocation of transcription factor TF-Z and increased CYPZ transcription. A loss-of-function mutation in the phosphatase P reduces dephosphorylation of the kinase in the cascade, increasing its active time. You are provided with the following experimental observations from hepatocyte cultures:

- Wild-type baseline CYPZ mRNA: 400 arbitrary units (AU).
- After hormone stimulus, wild-type CYPZ mRNA increases to 1600 AU at 2 hours, then returns to 450 AU at 8 hours.
- In P loss-of-function mutant, baseline CYPZ mRNA is 600 AU; after same stimulus CYPZ mRNA increases to 3200 AU at 2 hours and is 1800 AU at 8 hours.
- Drug clearance in mice is proportional to CYPZ activity; in wild-type clearance rate doubles transiently after hormone and returns close to baseline; in mutant mice clearance increases fourfold and remains elevated for longer.

(a) Explain, with reference to phosphorylation state and TF-Z dynamics, why the P loss-of-function mutant has higher baseline CYPZ mRNA than wild-type. **(4)**

(b) Calculate the fold induction at 2 hours in wild-type and mutant cells (use stimulated level / baseline). Give answers to 2 significant figures. **(4)**

(c) Using the 8 hour values, calculate the percent remaining of the peak (2 hour) CYPZ mRNA at 8 hours for wild-type and mutant. Give answers to 3 significant figures. **(4)**

(d) Propose a simple kinetic explanation, using rates of TF-Z nuclear import and removal, for why the mutant shows both greater peak induction and slower return toward baseline. Use labelled rate terms in your answer. **(8)**

(e) Considering the drug clearance observations in mice, evaluate the likely clinical consequence of P loss-of-function for dosing of the drug metabolised by CYPZ. Include at least two distinct consequences and a brief suggestion for therapeutic monitoring or dose adjustment. **(11)**

(Total for Question 10 is 31 marks)

Mark scheme · AB8D The Control of Gene Expression: Depth and Exam Drill

Question 1

- (a) **B1** promoter is a DNA sequence where RNA polymerase and associated factors bind to initiate transcription oe
- **(a) Answer: A DNA sequence where RNA polymerase and factors bind to start transcription.**
- (b) **B1** operator is a DNA site adjacent to a promoter where regulatory proteins such as repressors bind to control transcription oe
- **(b) Answer: A DNA site near the promoter where regulatory proteins such as repressors bind to control transcription.**

Question 2

- **Level 3** (5-6): Detailed explanation that links repressor binding, allolactose binding, operator accessibility and resulting transcription with clear causal statements and correct terminology.
- **Level 2** (3-4): Reasonable explanation that includes most key points but lacks some causal clarity or precise terminology.
- **Level 1** (1-2): Simple statement that mentions either repressor or inducer without linking to control of transcription.
- **Indicative content:**
  - In absence of lactose the lac repressor binds to the operator preventing RNA polymerase from transcribing lac genes; this stops production of lactose-metabolising enzymes.
  - Allolactose, an isomer of lactose formed at low rate, acts as an inducer by binding to the repressor and causing a conformational change.
  - Binding of allolactose reduces the repressor affinity for the operator so it dissociates, freeing the promoter for RNA polymerase binding.
  - RNA polymerase can then transcribe the lacZYA genes producing beta-galactosidase, permease and transacetylase; gene expression increases only when inducer is present.
  - Details of negative control, reversible binding, and the energetic advantage of expressing enzymes only when substrate is available.

Question 3

- (a) **M1** set rate of change 0 = R - k Nss or equivalent steady state equation
- (a) **A1** Nss = R / k cao
- **(a) Answer: Nss = R / k**
- (b) **M1** calculate k = ln 2 / 5.0 = 0.138629... min^{-1} or equivalent
- (b) **M1** substitute Nss = 120 / 0.138629... oe
- (b) **A1** 866 transcripts (3 s.f.), cao
- **(b) Answer: 866 transcripts**
- (c) **M1** calculate new k = ln 2 / 2.0 = 0.346574... min^{-1}
- (c) **M1** calculate new Nss = 120 / 0.346574... and fold change = new/old, oe
- (c) **A1** new Nss = 346 transcripts and fold change = 0.400 (3 s.f.), cao
- **(c) Answer: 346 transcripts; fold change 0.400**

Question 4

- (a) **M1** recognise that fraction bound 0.5 occurs when [TF] = Kd for this binding equation
- (a) **M1** interpolate between values: at 5 nM fract 0.43 and at 10 nM fract 0.67 so Kd between 5 and 10 nM
- (a) **A1** Kd approx 6.5 nM, cao
- **(a) Answer: Kd = 6.5 nM**
- (b) **M1** substitute fraction = 2/(6.5 + 2) = 2/8.5 = 0.235...
- (b) **M1** calculate percent error = (measured - model)/measured x 100 or equivalent and evaluate
- (b) **A1** calculated fraction = 0.24; percent error = (0.20 - 0.235)/0.20 x100 = -18% approx, cao
- **(b) Answer: Calculated fraction = 0.24; percent error = -18%**
- (c) **B1** loss of activity of TF1 at low concentration due to adsorption to tube walls or partial denaturation
- (c) **B1** incomplete equilibration or slow association kinetics so not enough complex formed during incubation
- (c) **B1** non-specific binding or probe degradation reducing available labelled probe, lowering apparent fraction bound
- **(c) Answer: Possible reasons: loss of active TF1 at low concentration (adsorption/denaturation); incomplete equilibration/slow association kinetics; non-specific binding or probe degradation reducing available labelled probe.**

Question 5

- (a) **M1** recognise per-cycle factor is 1.9 and expression is $N = 1 \times (1.9)^n$ or equivalent
- (a) **A1** $N = (1.9)^n$, cao
- **(a) Answer: $N = (1.9)^n$ copies**
- (b) **M1** evaluate $(1.9)^{30}$ correctly or using log, oe
- (b) **M1** convert to scientific notation correctly
- (b) **A1** answer 2.30×10^8 (3 s.f.)
- (b) **A1** unitless count or 'copies' accepted, cao
- **(b) Answer: 2.30×10^8 copies**

Question 6

- (a) **M1** substitute percent decrease = (0.62 - 0.41)/0.62 x100
- (a) **A1** 34% (2 s.f.), cao
- **(a) Answer: 34%**
- (b) **M1** substitute SEdiff = $\sqrt{0.03^2 + 0.02^2}$
- (b) **A1** 0.036 (2 s.f.)
- **(b) Answer: 0.036**
- (c) **M1** calculate difference = 0.21 and twice SEdiff = 0.072, oe
- (c) **A1** 0.21 >> 0.072 so change is likely statistically significant at about 95% level, cao
- **(c) Answer: Difference 0.21 > 2 x SEdiff 0.072, so likely statistically significant at about 95% level.**

Question 7

- (a) **B1** missense: single base change that alters a codon so it codes for a different amino acid oe
- (a) **B1** nonsense: mutation that changes a codon to a stop codon, causing premature termination of translation oe
- **(a) Answer: Missense: a single base change that alters a codon so a different amino acid is incorporated. Nonsense: a mutation that converts a codon to a stop codon, causing premature termination.**
- (b) **M1** calculate number of complete codons before stop: codons = floor((601 - 1)/3) + 1? or simpler: nucleotide position 601 is in codon number 201, oe
- (b) **A1** truncated protein length = 200 amino acids (if stop at codon 201, only 200 full amino acids before stop), cao
- **(b) Answer: 200 amino acids**

Question 8

- **B1** repressible operons are usually on by default and turned off by the corepressor binding to repressor (example: trp operon), oe
- **B1** inducible operons are usually off by default and turned on by an inducer binding to repressor or activator (example: lac operon), oe
- **B1** mechanism difference: repressible uses negative feedback when end product high; inducible responds to presence of substrate to allow expression
- **B1** functional consequence: repressible prevents wasteful synthesis when product abundant; inducible allows rapid response to available substrate
- **Answer: Repressible operons are usually active and shut down when a corepressor binds the repressor (negative feedback); inducible operons are usually inactive and activated when an inducer prevents repressor binding or recruits activator. Repressible saves resources when product abundant; inducible allows expression only when substrate present.**

Question 9

- (a) **M1** calculate replicate normalisations: 1200/800=1.5; 1350/900=1.5; 1280/850=1.50588...
- (a) **M1** calculate mean = (1.5 + 1.5 + 1.505882)/3
- (a) **A1** mean control = 1.50 (3 s.f.), cao
- **(a) Answer: Mean normalised control = 1.50**
- (b) **M1** calculate treated normalisations: 760/820=0.92683; 690/810=0.85185; 720/830=0.86747
- (b) **A1** mean treated = 0.882 (3 s.f.), cao
- **(b) Answer: Mean normalised treated = 0.882**
- (c) **A1** 59% (2 s.f.), cao
- **(c) Answer: 59%**

Question 10

- (a) **M1** state that phosphatase P normally dephosphorylates an active kinase, limiting its active time
- (a) **M1** loss of P increases steady-state level of phosphorylated active kinase
- (a) **M1** more active kinase leads to increased phosphorylation and nuclear translocation of TF-Z even without stimulus
- (a) **A1** this causes higher baseline transcription of CYPZ in the mutant, cao
- **(a) Answer: Without P activity the kinase remains more phosphorylated and active, increasing TF-Z phosphorylation and nuclear localisation even in absence of stimulus; increased TF-Z in nucleus enhances basal CYPZ transcription, explaining higher baseline mRNA.**
- (b) **M1** wild-type fold = 1600/400 = 4
- (b) **M1** mutant fold = 3200/600 = 5.333...
- (b) **A1** wild-type 4.0 (2 s.f.)
- (b) **A1** mutant 5.3 (2 s.f.), cao
- **(b) Answer: Wild-type 4.0; Mutant 5.3**
- (c) **M1** wild-type percent = 450/1600 x100
- (c) **M1** mutant percent = 1800/3200 x100
- (c) **A1** wild-type = 28.1% (3 s.f.)
- (c) **A1** mutant = 56.3% (3 s.f.), cao
- **(c) Answer: Wild-type 28.1%; Mutant 56.3%**
- (d) **M1** define rate of TF-Z nuclear import as k_{in} and rate of TF-Z removal (export or dephosphorylation) as k_{out}
- (d) **M1** state that phosphatase P loss reduces k_{out} or increases effective k_{in}/k_{out} ratio
- (d) **M1** higher k_{in}/k_{out} leads to larger steady-state nuclear TF-Z during stimulus hence greater transcriptional activation and larger peak
- (d) **M1** reduced k_{out} slows removal of TF-Z from nucleus after stimulus, causing slower decline of transcription and higher remaining mRNA at 8 hours
- (d) **M1** link to mRNA dynamics: transcription rate is proportional to nuclear TF-Z, and mRNA decay means prolonged high transcription sustains higher mRNA
- (d) **M1** mention that kinase dephosphorylation being slower increases active kinase lifetime, effectively decreasing k_{out} or increasing TF-Z phosphorylation rate
- (d) **M1** explain qualitative prediction: mutant has both increased peak and prolonged tail in mRNA timecourse
- (d) **A1** coherent kinetic chain described linking molecular rates to observed mRNA timecourse, cao
- **(d) Answer: Let k_{in} be TF-Z nuclear import rate and k_{out} its removal rate. Loss of P reduces k_{out} (or increases effective phosphorylation-driven import), increasing k_{in}/k_{out} so more TF-Z accumulates in nucleus during stimulus giving larger peak transcription. Slower k_{out} delays TF-Z removal after stimulus so transcription stays elevated longer; with mRNA decay this produces a slower return of CYPZ mRNA to baseline.**
- (e) **M1** state that increased CYPZ expression increases metabolic clearance of the drug
- (e) **M1** note that mutant shows fourfold increase in clearance and prolonged elevation, so drug exposure (AUC) will be substantially reduced
- (e) **M1** consequence 1: reduced therapeutic efficacy at standard dose because plasma concentration falls faster
- (e) **M1** consequence 2: need for higher or more frequent dosing to maintain therapeutic levels, with risk of variable response
- (e) **M1** risk: induction can persist so accumulation of active metabolites or production of toxic metabolites could occur; mention possibility
- (e) **M1** suggest monitoring plasma drug concentration or pharmacodynamic markers to guide dose adjustment
- (e) **M1** suggest alternative strategy: use drugs not metabolised by CYPZ or co-administer CYPZ inhibitor cautiously
- (e) **M1** comment on safety: faster clearance might require higher dose but also increases risk if the drug has active metabolites or narrow therapeutic index
- (e) **M1** state that genetic testing for P loss-of-function could inform personalised dosing
- (e) **M1** weigh benefits and harms and conclude that increased dosing may be needed but must be guided by monitoring, cao
- (e) **A1** clear, balanced evaluation with monitoring and alternative suggestions earning full credit

- **(e) Answer: Likely consequences: CYPZ induction increases clearance so standard dosing will give lower drug exposure and reduced efficacy; sustained induction in mutant causes prolonged low exposure necessitating higher or more frequent dosing. Risks include altered metabolite profiles and toxicity if metabolites are active. Recommended actions: therapeutic drug monitoring of plasma concentrations or PD markers, consider genotype-guided dosing, or choose alternative drugs not metabolised by CYPZ. Any dose increase should be cautious and guided by monitoring.**