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Molecular Biology and Biochemistry

694:408 115:512

Spring 02

Exam #1: Transcription

1. (10 Points) Take home problem on database analysis of plasmid insert Please attach your printouts to test and have your name on them. Late hand ins will not be accepted.

- a) Brown Sporulation Microarray (3pts) _____
b) YPD questions (7 pts) _____

For questions 1-21 there is only one correct answer for each question; place it on the answer sheet on page 1. Read each statement carefully. Each of these questions is worth 2 points

2. Mutations in the -35 region of the *lac* operon would
- express the lac repressor constitutively
 - block the binding of RNA polymerase to the promoter
 - express β -galactosidase constitutively
 - prevent the inducer from binding to the repressor
 - all of the above
3. An *E coli* cell that has the genotype $I^+ O^c Z^- / I^+ O^+ Z^+$ would
- repress β -galactosidase synthesis upon the addition of lactose
 - constitutively synthesize β -galactosidase
 - be unable to synthesize β -galactosidase
 - only induce β -galactosidase synthesis upon the addition of lactose
 - none of the above
4. CAP interacts with which of these factors?
- DNA
 - AraC
 - cAMP
 - CTD
 - all of the above
5. Which of the following is not used in the S1 assay?
- a radiolabeled DNA fragment
 - a polyacrylamide gel
 - a DNA-binding protein
 - S1 nuclease
 - a. and b.

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6. The footprinting assay can be used to
 - a. map the position of nucleosomes in chromatin
 - b. determine the level of expression of a specific transcript
 - c. determine the DNA-binding site for a transcription factor
 - d. map the ends of a transcript
 - e. a. and c.

7. The structure of RNAP is similar to a?
 - a. Helix-turn-helix
 - b. Leucine zipper
 - c. Histone fold
 - d. Crab claw
 - e. Zinc finger

8. Binding of an activator protein to DNA can activate RNAPII transcription by
 - a. recruiting a HAT complex to the promoter
 - b. recruiting sigma to the promoter
 - c. recruiting TBP to the promoter
 - d. recruiting SL1 to the promoter
 - e. a and c

9. Which of the following proteins or complexes does not bind the promoter of a yeast *GAL* gene?
 - a. Gal4
 - b. TBP
 - c. TFIIB
 - d. CAP
 - e. a. and d.

10. Which of the following statements about the *trp* operon is false?
 - a. In the absence of tryptophan the entire operon is transcribed.
 - b. The Trp repressor prevents attenuation of transcription
 - c. The Trp repressor is activated by tryptophan
 - d. The leader peptide binds to the stem loop structure formed by the mRNA
 - e. b and d

11. Which of the following statements is true about an operon governed by negative control?
 - a. Such operons are OFF in the absence of their regulatory protein
 - b. The regulatory operator is required for turning ON the operon
 - c. Repressor bound to the operator prevents RNAP from functioning
 - d. b and c
 - e. none of the above.

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12. All of the following statements about the carboxy terminal domain (CTD) of RNA polymerase are true except
- CTD is bound by the Srb/Med complex
 - CTD has homology with the α subunit of bacteria RNAP
 - CTD is critical for viability
 - CTD is modified by components of TFIIH
 - b and d
13. The eukaryotic TATA box sequence
- is the target of TBP in SL1
 - works in combination with the -35 element
 - is present in the promoters of all genes in the organism
 - acts to position RNA polymerase II for transcription initiation
 - c. and d.
14. All of the following steps can serve as regulatory steps for prokaryotic gene expression except
- binding affinity of RNAP
 - isomerization to the open complex
 - attenuation
 - mRNA export
 - mRNA degradation
15. A helix-turn-helix motif
- contains a helical coiled region involved in dimerization.
 - is found in both bacterial and eukaryotic transcription factors
 - requires a zinc ion to bind DNA
 - is a histone-like fold
 - none of the above
16. Which of the following is the correct order of binding of general transcription factors to initiate mRNA transcription?
- TFIIIA, TFIIIC, TFIIIB, RNAPIII
 - TFIID, TFIIIB, TFIIIF, TFIIIE
 - TFIIIB, RNAPII, TFIIIE, TFIIH
 - TFIID, TFIIIC, TFIIIB, RNAPII
 - b and c
17. What is the function of TFIIH in the transcription initiation complex?
- TFIIH binds to the TATA box
 - TFIIH helps stabilize TBP binding
 - TFIIH is required for promoter clearance by RNAP
 - TFIIH phosphorylates TFIID
 - b. and d.

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18. The TFIID complexes

- a. always contains the same set of TAFs
- b. contain some common and some different TAFs
- c. is not essential for transcription of mRNA
- d. contains TBP and is identical to the SL1 complex
- e. are not a target for activator proteins

19. The Rho factor does not

- a. bind mRNA
- b. form a hexamer complex
- c. require a stem-loop structure to function
- d. have ATPase activity
- e. a and c

20. RNAPI, RNAPII and RNAPIII

- a. contain exactly the same subunit proteins
- b. do not contain any common subunit proteins
- c. share some common subunits but also have different subunits
- d. have subunit proteins that are not similar to bacterial RNAP
- e. a and d

21. The N-terminal tails of histones

- a. are acetylated
- b. are methylated
- c. are phosphorylated
- d. are involved in transcriptional regulation
- e. all of the above

For the remaining questions, write them in the space provided. Please strive for short answers in clear hand writing

22. Name four DNA binding motifs (4 points)

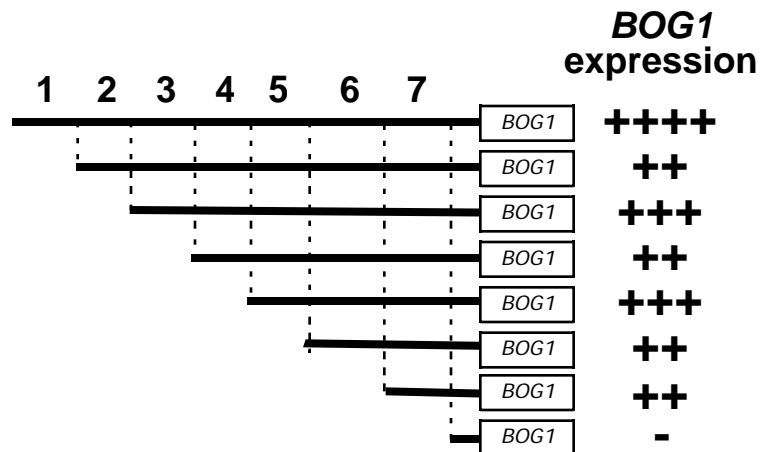
23. Name three assays used to monitor DNA binding by a protein (3 points)

24. Mutations were isolated in three different regions of the genome, 1, 2 and 3, that affect transcription of the gene XYZ. Mutant 1 contains a mutation 50 bp upstream of the XYZ gene which prevents RNAP from binding to the promoter. Mutant 2 contains a mutation 400 bp downstream of XYZ which introduces an amino acid substitution that inactivates a protein required for transcriptional activation of XYZ. Mutant 3 contains a mutation that is over 20 kb from XYZ which inactivates a binding site for the transcriptional activator protein. Which of these mutants are cis acting and which are trans acting? How would you test this? (6 points)

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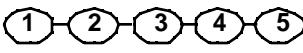
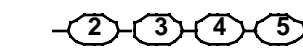
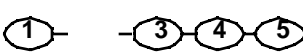
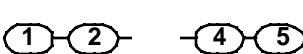
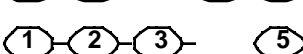
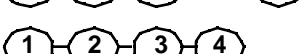
25. What is combinatorial control of transcription? What are its advantages? (5 points)
26. Which step in the process of transcription is the most common one to be regulated to control gene expression? Why? (6 pts)
27. What are the differences between the HAT and HDAC complexes? What are their respective roles in transcription? (3 points)
28. Why does the mechanism of transcriptional attenuation of the *trp* operon not function in human cells? (3 points)
29. A student is interested in the transcriptional regulation of the yeast *BOG1* gene. To identify the different elements in the promoter that are required for transcription she makes a series of seven deletions in the promoter and wants to assay transcription of these mutants. A) List 4 ways she can use to monitor the level of expression from each of the mutant promoters (4 points)

She monitors expression of each of the mutants and gets the following results. List the apparent function of each of the regions in the promoter. (6 points)



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30. A student is interested in investigating the different domains of Zub1, a transcriptional regulatory protein that binds cooperatively with the Zub2 transcription factor to elements in the promoter of the *BOG3* gene to activate its transcription. Zub1 also binds cooperatively with the Zub4 protein to activate transcription of the *BOG5* gene. She makes a series of deletions in the gene coding for Zub1, purifies the protein, and assays for cooperative DNA binding in complex with Zub2 and with Zub4. +++ indicates wild type levels of binding activity. A - indicates no detectable binding. She then transforms the mutants into cells and assays for the level of *BOG3* and *BOG5* expression. She knows that in the presence of Zub1 she gets full expression of *BOG3* and *BOG5* (indicated by +++) and in the absence of Zub1 she gets no expression (-). What can she conclude about the function of the five regions of the protein? (10 points)

Clone #	Domain Deleted		Cooperative DNA-binding with Zub2	Cooperative DNA-binding with Zub4	Activation of <i>BOG3</i>	Activation of <i>BOG5</i>
1	WT		+++	+++	+++	+++
2	Δ1		+++	—	+++	—
3	Δ2		—	—	—	—
4	Δ3		—	+++	—	+++
5	Δ4		+++	+++	—	—
6	Δ5		+++	+++	—	+++

Domain 1 _____

Domain 2 _____

Domain 3 _____

Domain 4 _____

Domain 5 _____

Answers to non-multiple choice questions:

22. Helix-turn-helix, Leu zipper, Zinc finger, helix-loop-helix
23. EMSA, Footprinting (chemical and enzymatic), in vivo reporter assays, chromatin immunoprecipitation assays (ChIPs)
24. Mutant 1 is a cis acting mutation in the promoter of the gene. It prevents binding of RNAP. Mutant 2 is a trans-acting mutation in a regulatory protein that is required for activation of the gene. Mutant 3 is a cis-acting mutation because it inactivates a binding site that is required for activation of the gene. To test this transform in wild type versions of each mutant with a mutant XYZ gene. A wild type copy of the mutant 2 gene will

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complement the mutant copy. Wild type copies of the cis-acting mutants 1 and 3 will not be able to complement the mutants.

25. Combinatorial control allows for more variability of different regulatory activities with fewer regulatory factors. Interactions of a given regulatory protein with other proteins allows it to bind to different regulatory sites and therefore regulate different sets of genes. These different protein interactions can also determine if the protein functions as a repressor or an activator.
26. Although transcription can be regulated at many different steps, the recruitment of the TFIID complex appears to be the major form of regulation.
27. HAT – Histone acetyltransferase – acetylates histone tails which is associated with transcriptional activation of the target genes. An HDAC is a histone deacetylase complex which removes acetyl groups from the histone tails. Hypo-acetylation often correlates with a repressed state of transcription of the gene.
28. Transcriptional attenuation of the trp operon in prokaryotes is caused by the failure of ribosome pausing at a specific point on the transcript, allowing a stem-loop structure of the mRNA to form, signaling transcription termination. In eukaryotes, transcription occurs in the nucleus and translation in the cytoplasm. The two processes are therefore not physically connected as they are in prokaryotes. Transcription of the message is complete before the message is translated.
29. Northern blots, S1 protection experiments, primer extensions, lacZ reporters. Note: Microarray is **not** an acceptable answer here. The student wants to look at the effect of particular cis-acting mutations on the regulation of one promoter. Microarray analysis is used to examine the expression on a genome-wide basis. These experiments are considerably more expensive (\$1,000 per assay) and time consuming (several days) than a lacZ assay or Northern blots.
30. 1. Activator element; 2. Repressor element; 3. Activator element; 4. Repressor element; 5. Activator element; 6. No apparent function; 7. Activator or general promoter element (TATA box)
31. Domain 1 - Involved in protein interactions with Zub4;
Domain 2 - DNA-binding domain
Domain 3 - Involved in protein interactions with Zub2
Domain 4 - Activator domain for both *BOG3* and *BOG5*
Domain 5 – Activator domain for only *BOG3*

The Zub1 protein binds cooperatively with Zub2 and interacts with two different co-factors (suggested by both domains 4 and 5 are required) to activate expression of *BOG3*. It binds cooperatively with Zub4 and only interacts with one co-factor (suggested by only domain 4 being required) to activate expression of *BOG5*.