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MCB 110

Spring 2002

Midterm exam II

April 7, 2003

Write your name and Student ID# on all pages. Only exams written in non-erasable ink pen will be considered for regrading. 150 points total.

Question 1	40 points	34
Question 2	25 points	19
Question 3	35 points	33
Question 4	30 points	10
Question 5	20 points	15
Total		110

SHORT ANSWERS ARE ENCOURAGED; POINTS WILL BE SUBTRACTED FOR WRONG ANSWERS EVEN IF THE CORRECT ANSWER IS ALSO PROVIDED. THE SPACE PROVIDED ON THE FRONT PAGE SHOULD BE MORE THAN SUFFICIENT FOR A COMPLETE ANSWER.

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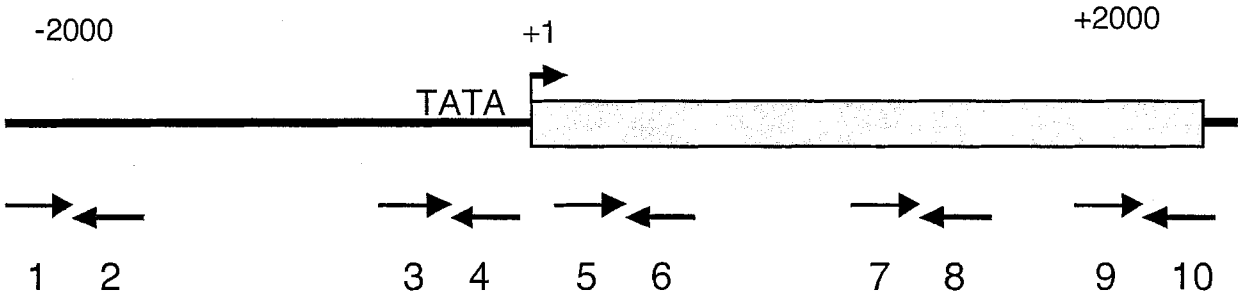
Question 1. (40 points total). You are studying transcriptional activation of the yeast HOT1 gene, which is reported to be induced by high growth temperatures.

Below is a diagram of the HOT1 gene. The start site of transcription is illustrated by the "+1" label. You have DNA oligonucleotides to use as primers that are illustrated below, numbered 1 through 10.

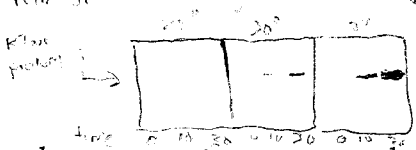


(Part A, 9 Points) You first need to confirm that transcription of the HOT1 gene is induced in yeast under conditions in your own laboratory. You have growth incubators at temperatures of 25, 30 and 37°C. Using the primers in the diagram, what experiment would you do?

8

Primer-extension

I would use labeled primer 10 to determine if transcription was occurring at each temperature using By reacting the extracted MRNA from the yeast w/ the primers and reverse transcriptase I could determine if transcription was occurring over time by running the products in a gel. If the reverse transcriptase reaction is successful then a single band will grow darker in subsequent lanes over time due to Klenow activity on the transcript DNA



For the next section, assume that you indeed confirm that transcription of HOT1 is induced by increasing the temperature. You are now interested in determining how the distributions of proteins along the HOT1 gene are altered during transcriptional activation.

(Part B, 10 Points) You are provided with antibodies that specifically recognize the TBP protein. When and where do you expect enrichment of TBP along the DNA in the diagram? Why do you have this expectation, and how will you test your ideas?

10

I would expect enrichment of TBP at the TATA box on the diagram because TBP binds to the TATA box and does not travel w/ the polymerase during elongation. I would test this w/ a ChIP assay using each of the primer pairs possible (i.e 1,2 ; 3,4, 5,6 etc.) in the PCR reaction. If only the DNA region between 3 and 4 shows up under autoradiography then I would know that the TBP binds at the TATA box and stays there.

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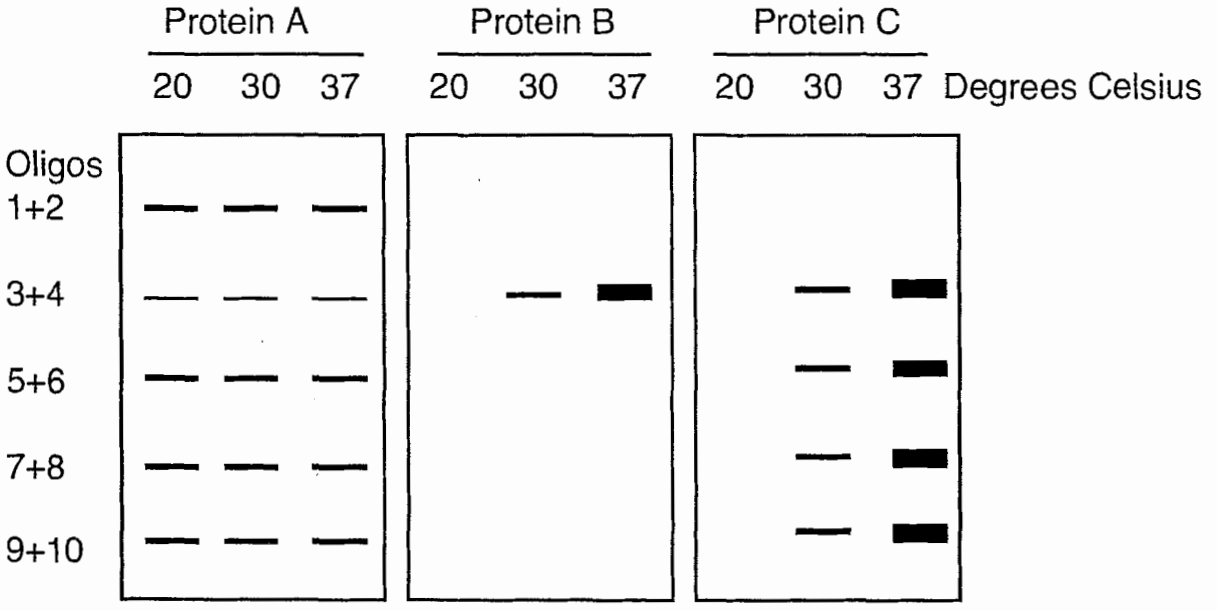
You are provided with antibodies that specifically recognize three proteins, called A, B and C. To investigate how these protein might contribute to the regulation of HOT1 transcription, you perform chromatin immunoprecipitation experiments with these antibodies, using cells grown at the indicated temperatures and the oligonucleotides from the previous diagram.



(Part C, 21 Points) Based on these data, propose a role for each of these three proteins during transcription of HOT1. For each of these, name one protein that we discussed in class that would display these patterns.

Protein A - Protein present regardless of transcription on all segments of DNA that can sometimes act as a repressor of transcription. Histones fall under this class of proteins

Protein B - TBP containing protein that binds at higher temperatures as an activator of transcription. Similar to TFIID/TBP complex general transcription factor for RNA pol II that doesn't follow pol II during elongation, stays at TATA box

Protein C - protein that covalently modifies nucleosomes making the DNA available for transcription. Example: HAT protein such as the SAGA complex

Student Name and SID# Eddie Vang 1545324

Question 2. (25 points total) To better understand transcriptional activation of the HOT1 promoter, you now perform mutational analysis. You make the following "linker scanning" mutations in the promoter, and fuse these constructs to the bacterial lacZ gene, as shown below. The hatched boxes indicated the regions of scrambled sequence for each DNA molecule; DNA #1 contains a completely wild-type promoter sequence.

Upon introduction of these DNA molecules into yeast, you observe the indicated expression pattern of β-galactosidase at the indicated temperatures.

DNA	Temperature, degrees Celcius	β -galactosidase
1	20	-
	30	+
	37	+++
2	20	-
	30	+
	37	+++
3	20	-
	30	+
	37	+
4	20	-
	30	+
	37	+++
5	20	-
	30	-
	37	-

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(Question 2A, 10 points) What do you conclude about the structure of the HOT1 promoter?

Conclude that the promoter has two important domains for transcriptional activation between bases -400 and -300 and between bases -200 and -100

6

(Question 2B, 15 points) You have already isolated active yeast RNA polymerase II and the general transcription factors. To better understand HOT1 activation, you fractionate yeast nuclear extracts and test for site-specific transcriptional activation from the HOT1 promoter *in vitro* as a naked DNA template. You discover that two different fractions can stimulate transcription. What kind of proteins do you predict are present in these fractions that are responsible for this activity? How would you test your predications?

I imagine the proteins are activators that bind to the enhancer or upstream activating sequences to promote transcription. I would test this hypothesis using DNA footprinting to see if proteins in the fractions were binding to DNA. I would also try to isolate the proteins' DNA binding binding domains or the region of DNA itself to see if they activate transcription, independent of DNA binding

4 4

Student Name and SID# Eddie Wang 15453214

Question 3. (35 points total) While on spring break, you isolate a new bacterial (prokaryotic) species from the waters of Lake Merritt. You name this organism *Bacillus merrittosis* and find that it is able to metabolize a carbon source unique to its environment, a complex polymer of glucose called merrittose.

To study the regulation of merrittose metabolism, you isolate mutants unable to grow in the presence of merrittose, but able to grow in the presence of glucose.

(Question 3A, 20 points, 5 points each). Propose four different kinds of mutations that would result in this phenotype. If any of your answers are not recessive mutations in trans-acting factors, describe these aspects.

(1) Mutation of gene that allows merritose to enter the bacteria (permease), a defective permease would allow no merritose to enter and be metabolized

(2) Mutation of gene that cleaves merritose to glucose units, a defective gene like this would prevent metabolism

(3) If genes are positively regulated, mutation of the activator's DNA binding region on the DNA would prevent activator binding so there would be no transcription of the metabolic genes.

(4) Mutation in activator proteins gene could prevent it from binding to DNA and thus prevent transcription

Your lab partner has isolated several different *B. merrittosis* mutants unable to metabolize a different carbohydrate called cerritose. You discover that introduction of an F' plasmid containing a gene called MerA can restore the ability of one of your mutants to grow on merritose and one of your partners' mutants to grow on cerritose. No other single gene can restore prototrophy (wild-type metabolism) to <u>both</u> mutants.

(Question 3B, 15 points). Propose a mechanism of action of the MerA protein. Name one biochemical property you expect for MerA, and propose how you will test your idea.

If the MerA gene is similar to CAP in E.coli then binding of MerA to a ligand such as cAMP could allow it to bind to the promoters of the genes for both merritose and cerritose metabolism, acting as a positive regulator for both if they are part of one regulon

Could use DNA footprinting on the promoter regions of both cerritose and merritose genes in the inducible mutants in the presence and absence of MerA to see if it is binding to their promoter regions. Could use Northern blotting to see if their gene transcripts are being made. In each case

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Question 4. (30 points total) You are investigating the differences between transcriptional activation on naked DNA versus chromatin templates, using a simple promoter with a single upstream site for the Sp1 protein we discussed in class. Transcription on the naked DNA template requires RNA polymerase II, the general transcription factors, and Sp1, but transcription from the chromatin template requires two additional chromatographic fractions.

(Question 4A, 15 points) Your lab partner hypothesizes that the two activating fractions contain different histone acetytransferases that modify different residues on different core histones. Describe experiments you would perform to test these ideas.

Place chromatographic fractions in separate tubes, Add radioactively labeled acetyl-CoA in with each along with the chromatin template and the protein from the first test in both test tubes,

Isolate the chromatin from the tubes and separate the core histones. Measure the presence of acetylation on each amino acid using Edman degradation and liquid scintillation radioactivity measurements. This will show which amino acids have been acetylated on the histones by any HATs present in each fraction,

- How?

10

(Question 4B, 15 points) It turns out that your lab partner is only partially correct, only one of your activating fractions contains histone acetyltransferase activity. What do you hypothesize is in the other fraction required for transcription on the chromatin template? Describe a biochemical assay for your proposed protein, and any additional reagents you would need for this.

The second activating fraction could be a chromatin remodeling complex such as SWI/SNF that is altering the nucleosome positions. The activity of a chromatin remodeling complex can be assayed by a gel shift electrophoretic mobility assay. This would require radioactively labeled chromatin along w/ the chromatographic fraction. If the nucleosomes are being moved by the complex it will show up as a gel-shift.

The second activating fraction could be an activating protein whose activating domain recruits the HAT containing complex from the other fraction. The binding of this protein could be assayed by DNase footprinting w/ a labeled region of DNA from the upstream region.

Student Name and SID# Eddie Wang 3643214

Question 5. (20 points total, 5 points each) You make a extract from human cells, removing soluble proteins and discarding membrane-bound proteins. You then separate the soluble macromolecules by sucrose gradient sedimentation. Which of the following statements are true? Why or why not in each case?

A. The acetyltransferase activities near the bottom of the gradient will preferentially acetylate nucleosomes rather than free histones, because nucleosomes have greater mass than free histones.

True

B. Because of its greater positive charge, RNA polymerase II will be found closer to the bottom of the gradient than RNA polymerase I.

False, sedimentation is based on mass, not charge

✓

C. TBP will only be found in one region of the gradient, as part of the TFIID complex.

False TBP is also present in the general transcription factors of RNA pol I and III which could be in different regions.

✓

D. You won't be able to study steroid receptors in this experiment, because they will have been discarded with the membrane-bound molecules.

False, steroid receptors are domains of non membrane-bound molecules such as the glucocorticoid receptor

✓