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BIOCHEMISTRY 353, SPRING 2002 SECOND HOUR EXAM, MARCH 6, 2002

Before you start, **PRINT your name on each page**. Be sure to print your name at the top of each page. Notes of any kind are NOT permitted.

Confine your answers to the space provided. Only answers in the space provided will be graded. If you cross out an entire answer, you may write your new answer in the same amount of space on the back of the page. Be sure to clearly state in a box on the front of the page that the answer is on the back of the page, or your answer will not be seen by the grader.

THIS EXAM IS 10 PAGES LONG. BEFORE STARTING CHECK THAT YOU HAVE ALL OF THE PAGES

DO NOT TURN THIS PAGE OVER UNTIL INSTRUCTED TO DO SO BY A PROCTOR

Part I _____/30

Part II _____/30

Part III _____/18

Part IV _____/22

TOTAL _____/100

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Part I. TRUE-FALSE (30 points total, 3 points each) Circle T for statements which are True and F for statements that are False. (GRADED RIGHT MINUS WRONG; This means that if you got 8 right and 1 wrong and left 1 blank you would get $8 - 1 = 7 \times 3$ or 21 points.)

- T F 1. The DNA sequences of the human and *Xenopus* (a cold blooded amphibian) estrogen receptor DNA binding domains differ by more than 10%. This means that the amino acid sequences of the DNA binding domains of the human and *Xenopus* estrogen receptors are completely unrelated to each other.
- T F 2. The DNA binding domains of steroid hormone receptors contain bound zinc.
- T F 3. DNase I footprinting is a method used to identify the specific binding site on DNA of a protein, such as a repressor.
- T F 4. Eukaryotic RNA polymerase II has a subunit called sigma or sigma factor that greatly facilitates binding of RNA polymerase II to the TATA box.
- T F 5. In transcription by *E. coli* RNA polymerase, the newly synthesized RNA chain grows in the 5'→3' direction.
- T F 6. A mutation in eukaryotic RNA polymerase II that prevented the "clamp" from closing would most likely result in synthesis of RNA transcripts that were much shorter than normal.
- T F 7. In the histone code model, when the N-terminal regions of the histones are unmodified, proteins that function to perpetuate gene silencing recognize the unmodified histone tails, bind to the histone tails and help to perpetuate gene silencing near their binding site.
- T F 8. A single-stranded DNA oligonucleotide is just as effective as a double stranded siRNA in inducing degradation of a complementary mRNA.
- T F 9. Because the lac repressor has such a low affinity for its binding site, it is present at extremely high levels in *E. coli*.
- T F 10. If written as double stranded DNA, the DNA sequence 5'-AGGTACAGGTAC-3' is a perfect palindrome.

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Part II. Multiple Choice Questions (30 points 3 points each) Each question has ONE best answer. Circle the ONE letter best corresponding to the correct answer for each question. There is no penalty for guessing wrong.

1. Which of the following statements about the regulation of eukaryotic gene expression is NOT correct (in other words is false).

- A. Transcription does NOT involve promoters.
- B. Activation of transcription by bound transcription factors is more common than active repression by bound transcription factors.
- C. Transcription and translation are usually physically separated.
- D. Transcription activation involves the reorganization of chromatin structure.
- E. All of the above statements about eukaryotic transcription are correct.

2. Which of the following is a DNA sequence?

- A. Activation Function 1.
- B. Coactivator.
- C. Histone deacetylase.
- D. All of the above are DNA sequences.
- E. None of the above are DNA sequences.

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3. Which of the following statements about transcription attenuation are true.
- A. Regulating gene expression by attenuation is the most common way cells respond to changes in intracellular cyclic AMP levels.
 - B. The leader peptide gene of the Tryptophan (*trp*) operon contains Tryptophan (*Trp*) codons.
 - C. The leader peptide encodes a ribonuclease (RNase) that degrades the newly synthesized *Trp* mRNA.
 - D. When RNA polymerase transcribes the leader peptide region of Tryptophan (*trp*) operon, the DNA changes conformation so that a second molecule of Trp repressor binds at this site, preventing additional polymerases from transcribing through this region of the operon.
 - E. All of the above statements about attenuation are TRUE.
4. Which of these statements about the lactose (*lac*) operon and its regulation are true when *E. coli* are being grown in a medium containing glucose, but NO lactose.
- A. The cyclic AMP binding protein (called CAP and CRP) is bound to the *lac* operator.
 - B. RNA polymerase binds to the *lac* promoter and efficiently transcribes the *lac* operon.
 - C. Lac repressor is bound to the *lac* operator.
 - D. The cyclic AMP binding protein (called CAP and CRP), displaces the Lac repressor from the *lac* promoter.
 - E. None of the above statements are true.

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5. Which of these statements about nucleosomes are correct.
- A. Nucleosomes were first discovered as the method that bacteriophage use to condense their DNA and then were observed in *E. coli*.
 - B. Nucleosomes are especially rich in acidic amino acids, such as aspartic acid.
 - C. Nucleosomes can only form after the histones in the nucleosome are acetylated.
 - D. Nucleosomes are always found in chromatin at irregular intervals along the DNA.
 - E. Histone H1 is not present in the core nucleosome octamer.
6. Which of these statements about the helix-turn-helix motif for recognition of specific DNA sequences are correct.
- A. In the helix-turn-helix motif one helix binds in the major groove of the DNA and the other helix binds in the minor groove of the DNA.
 - B. In the helix-turn-helix motif one helix binds to the DNA and the other helix is exposed on the surface of the protein where it binds coactivator and corepressor proteins.
 - C. Proteins containing the helix-turn helix DNA recognition motif can be activators of transcription or repressors of transcription.
 - D. The helix-turn-helix motif contacts DNA through a leucine zipper.
 - E. The helix-turn-helix motif is only found in eukaryotic DNA binding proteins.
7. The mechanism of the RNA polymerase II enzyme reaction is being worked out through biochemical and structural studies, but is not known with absolute certainty. Which of these statements about the role of magnesium in the reaction catalyzed by eukaryotic RNA polymerase II are likely to be correct.
- A. There is an active site magnesium between the nucleotide last added and the next nucleotide to be added.
 - B. There is a magnesium bound to the incoming rNTP.
 - C. After covalent bond formation and addition of the nucleotide to the RNA chain, magnesium is bound to the outgoing pyrophosphate.
 - D. None of these statements is likely to be correct.
 - E. All of these statements are likely to be correct

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8. How does chromatin that is undergoing active transcription differ from chromatin that is not undergoing transcription?
- A. Nucleosomes are absent or disordered in regions of very high transcriptional activity
 - B. The DNA in or near regions undergoing active transcription contains sites more sensitive to degradation by nucleases, such as DNase I.
 - C. The N-terminal histone tails are more highly acetylated in or near regions of the DNA undergoind rapid transcription.
 - D. All of the above statements are correct.
 - E. None of the above statements are correct
9. Which of the following statements about transcription initiation of eukaryotic genes transcribed by RNA polymerase II is correct
- A. Two of the three elements that help define starts sites for RNA polymerase II genes are usually sufficient to define a strong initiation site.
 - B. RNA polymerase II rarely has a defined start site for transcription, and initiates transcription anywhere in a tract of DNA approximately 200 nucleotides long.
 - C. The presence of a TATA box sequence in the DNA and the isolated purified TATA box binding protein (TBP) is sufficient for strong initiation of transcription near the TATA box sequence.
 - D. None of the statements is correct.
 - E. All of the statements are correct.
10. Which of these statements about eukaryotic RNA polymerases is correct.
- A. There are five major types of eukaryotic RNA polymerases.
 - B. The mushroom toxin α -amanitin inhibits all of the different classes of RNA polymerase equally well.
 - C. *E. coli* RNA polymerase has more subunits than any eukaryotic RNA polymerase.
 - D. If the C-terminal domain (CTD) repeats are deleted from RNA polymerase II, transcription will be greatly reduced.
 - E. None of the statements are correct.

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Part III. Fill in (18 points, 2 points each) There is no penalty for wrong answers. Only the first written answer will be graded. (There may be more than one correct answer)

1. If a eukaryotic DNA regulatory element can work at a large distance from the transcription start, can work upstream or downstream of the transcription start site and can work on any gene to which it is attached it is probably a member of the class of regulatory elements called _____.
2. Transcription by this eukaryotic RNA polymerase _____ (insert the number) is correctly initiated even if all of the sequence upstream of the transcription start site is deleted.
3. The protein, _____, is widely used as a reporter gene in transient transfections of eukaryotic cells.
4. Histone _____ is not found in the core histone octamer, and is probably absent in yeast.
5. To preferentially cut between the core and linker regions of histones, the enzyme _____ is often used
6. When this enzyme activity, _____, is inhibited by trichostatin A, the ability of histones bound to the DNA to repress transcription is decreased.
7. Both prokaryotic and eukaryotic promoters contain sequences important in the initiation of transcription that contain these two nucleotides _____.
8. How many different mRNAs are produced when the lactose operon is transcribed?

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9. Most transcription factors contain at least three types of domain. What are the names (do not use letters or numbers) of any TWO of these domains (1 point each)
- _____.

IV. Short Answer (22, points total, 4 points each, unless otherwise noted) Provide a brief biochemical explanation for each of the following observations or statements. Your answer should reflect the contents of this course. Only answers in the space provided will be graded, so think before you write. If you cross out an entire answer make a box indicating clearly that the answer is on the back, and use an equivalent amount of space on the back of the page. You do not have to fill in the entire space. If you know the answer, one or two short sentences or a simple diagram may be sufficient. If you keep writing, and write something that is incorrect, some credit will be deducted.

1. Highly purified preparations of *E. coli* RNA polymerase that have been passed over several columns during purification, efficiently transcribe nicked preparations of calf thymus DNA, but do not efficiently transcribe high molecular weight undegraded preparations of bacteriophage T₄ DNA. In contrast, crude extracts from *E. coli* efficiently transcribe high molecular weight undegraded DNA templates from bacteriophage T₄, but do not efficiently transcribe nicked preparations of calf thymus DNA.

2. In effort to silence the expression of a specific mRNA and eliminate the protein it codes for, a student attempted to carry out RNA interference (RNAi) on a cell line derived from humans. Since fairly short double stranded RNAs work well, the student assumed that a long RNA would work even better and used enzymes to copy both strands of the entire DNA sequence coding for the mRNA into a long double stranded mRNA. When this was inserted into the cells, they stopped making most proteins and quickly died.

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3. (6 points, 3 points each) A rho independent terminator contains a base paired stem rich in G:C base pairs followed by 6-10 uridines. To investigate the role of these sequence elements in termination a student made the following mutations. What would be the likely effect of these changes on the efficiency of termination at this site? Briefly explain your answer

A. Cytidines were substituted for DNA sequence coding for the 6-10 uridines.

B. Inserting 100 nucleotides of random sequence between the base paired G:C-rich stem and the tract containing 6-10 uridines

4. In a yeast 2-hybrid screen, a student prepared a library fusing a large pool of different cloned cDNA fragments to the Gal4 DNA binding domain. The known domain being tested for interaction with unknown proteins coded for by the library was fused to the Gal4 activation domain. The student was pleased to see that hundreds of independent and different clones were identified as positive for interaction with the known domain in the initial yeast 2-hybrid screen. When the student expressed these proteins and tested them one-by-one in biochemical assays, virtually none of them actually bound to the test domain.

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5. In a chromatin immunoprecipitation experiment a student was investigating whether a hormone ligand induced a steroid hormone receptor to bind to a possible recognition sequence at -330 from the transcription start site. The student cross-linked the DNA, sonicated it into fragments that averaged 600 nucleotides in length, immunoprecipitated the DNA-protein complexes with antibodies against the receptor, reversed the cross-links and amplified by PCR using primers complementary to sequences separated by 400 nucleotides that were in the center of the part of the gene transcribed to yield the mRNA. The student ran the gel, did not observe a PCR product at 400 nucleotides in the hormone-treated sample and concluded that the hormone ligand did not induce the receptor to bind to the potential regulatory site. A second research group using other methods concluded that the hormone-receptor complex did bind to the gene being studied and was later found to be correct. Assuming that the student executed the individual chromatin immunoprecipitation steps described above without ruining the experiment, where did the student go wrong. (in other words do not just write that the student lost the sample, forgot to turn on the PCR machine, the antibody didn't work etc. what is the error in conceiving or planing the experiment that the student made?)