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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 _____ X _____ /30

Part II _____ X _____ /30

Part III _____ X _____ /18

Part IV _____ X _____ /22

TOTAL _____ X _____ /100

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.)

- 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.
DNA binding domains of estrogen receptors are closely related, hence strong selective pressure, changes are in wobble bases. This exact question was gone over in lecture.
- T** 2. The DNA binding domains of steroid hormone receptors contain bound zinc.
- T** 3. DNase I footprinting is a method used to identify the specific binding site on DNA of a protein, such as a repressor.
- 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.
Sigma is in Prokaryotic RNA polymerase and the eukaryotic polymerase does not bind directly to the TATA box
- T** 5. In transcription by *E. coli* RNA polymerase, the newly synthesized RNA chain grows in the 5'→3' direction.
- T** 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.
This mutation will allow the RNA-DNA duplex to dissociate from the polymerase and terminate transcription prematurely. This will result in much shorter RNA transcripts.
- T** 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.
This is directly stated on the handout as the first item in the list and was gone over in the lecture.
- F** 8. A single-stranded DNA oligonucleotide is just as effective as a double stranded siRNA in inducing degradation of a complementary mRNA.

Since RNAi is based on a defense mechanism for double stranded viruses, single stranded DNA will be much less effective. Also mentioned in lecture was that double-stranded RNA is much more effective than single stranded RNA.

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*.

It has a very high affinity for its binding site and is present at very low levels in E. coli. In lecture we contrasted the high affinity of the lac repressor for the lac operator region with the much lower affinity of the CAP/CRP protein

F 10. If written as double stranded DNA, the DNA sequence 5'-AGGTCACAGGTCAC-3' is a perfect palindrome.

It is a direct repeat of AGGTCA

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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.

Answer: A

A is false, transcription does involve promoters

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.

Answer: E

3. Which of the following statements about transcription attenuation are true.
1. Regulating gene expression by attenuation is the most common way cells respond to changes in intracellular cyclic AMP levels.
 2. The leader peptide gene of the Tryptophan (*trp*) operon contains Tryptophan (*Trp*) codons.
 3. The leader peptide encodes a ribonuclease (RNase) that degrades the newly synthesized *Trp* mRNA.
 4. 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.
 5. All of the above statements about attenuation are TRUE.

B is 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.
1. The cyclic AMP binding protein (called CAP and CRP) is bound to the *lac* operator.
 2. RNA polymerase binds to the *lac* promoter and efficiently transcribes the *lac* operon.
 3. Lac repressor is bound to the *lac* operator.
 4. The cyclic AMP binding protein (called CAP and CRP), displaces the Lac repressor from the *lac* promoter.
 5. None of the above statements are true.

C glucose causes catabolite repression, no CAP/CRP bound to activate transcription.

5. Which of these statements about nucleosomes are correct.

1. Nucleosomes were first discovered as the method that bacteriophage use to condense their DNA and then were observed in *E. coli*.
2. Nucleosomes are especially rich in acidic amino acids, such as aspartic acid.
3. Nucleosomes can only form after the histones in the nucleosome are acetylated.
4. Nucleosomes are always found in chromatin at irregular intervals along the DNA.
5. Histone H1 is not present in the core nucleosome octamer.

E is correct

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.

C is correct. Only one helix binds the DNA, so A is false, the second helix stabilizes the first helix and is not usually exposed and is not a site for protein:protein interactions. The leucine zipper is a protein:protein interaction motif, not a protein:DNA interaction motif. Most of the proteins we discussed were prokaryotic proteins. CAP and Cro both contain this motif, one activates and the other represses transcription. This was stated explicitly in the lecture.

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

E. These statements are nearly verbatim from your book of lecture notes. The role of Mg was also discussed in the lecture..

8. How does chromatin that is undergoing active transcription differ from chromatin that is not undergoing transcription?

- 1. Nucleosomes are absent or disordered in regions of very high transcriptional activity
- 2. The DNA in or near regions undergoing active transcription contains sites more sensitive to degradation by nucleases, such as DNase I.
- 3. The N-terminal histone tails are more highly acetylated in or near regions of the DNA undergoing rapid transcription.
- 4. All of the above statements are correct.
- 5. None of the above statements are correct

D is correct

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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.

A is the only one that is 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.

D is the only statement that is correct

Name_____Key_____

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 **ENHANCERS** .
2. Transcription by this eukaryotic RNA polymerase **III, 3** (insert the number) is correctly initiated even if all of the sequence upstream of the transcription start site is deleted.
3. The protein, (**any one is okay**) **LUCIFERASE, Chloramphenicol Acetyl Transferase (CAT), β -Galactosidase, alkaline phosphatase**, is widely used as a reporter gene in transient transfections of eukaryotic cells.
4. Histone **1 or H1** 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 **Micrococcal nuclease** is often used
6. When this enzyme activity, **HDAC, histone deacetylase (NOT HAT, histone acetylase is incorrect)**, 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 ___ **T and A** (G and C are wrong, -1 for each wrong nucleotide +1 for each one correct)_____.

8. How many different mRNAs are produced when the lactose operon is transcribed?
 ___ **1** _____

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) **(DBD)**
DNA Binding Domain; transcription activation or activation function, TAF or AF;
protein:proteininteraction domain

(although not intended if you name specific features we will give credit, one of helix-turn helix, homeodomain, zinc finger; one of leucine zipper, basic helix loop helix ;

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.

(This is the experiment that led to the discovery of sigma factor. It was gone over in lecture.)

Nicked DNA from calf thymus (eukaryotic DNA, therefore lacks prokaryotic promoters and should not be efficiently transcribed by fully functional *E. coli* RNA polymerase). These data suggest that an easily dissociated factor present in the

crude extracts, but separated from the core RNA polymerase during purification, suppresses transcription on incorrect templates containing nicked DNA and enhances transcription on the phage promoters which are transcribed in *E. coli*.

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.

When long double stranded RNA molecules are introduced into a human cell they trigger a series of interferon-inducible pathways that involve specific protein kinases and result in both inhibition of translation of most mRNAs, not just the mRNA complementary to the double stranded RNA and induce non-sequence-specific degradation of a cell's mRNA (Fig. 1). This kills the cells. The key feature of siRNAs is that they do not activate the global interferon-induced pathway and result in sequence-specific degradation of the mRNAs complementary to the double stranded siRNA. By using the long double-stranded RNA the student activated this global pathway, translation ceased and the cells died.

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.

LESS TERMINATION

Since the Us in the sequence were replaced with C's there will be attract of Gs in the RNA and the G:C RNA:DNA duplex will be MORE stable than the A:U RNA :DNA

duplex. This will mean less chance of the duplex dissociating before the double-stranded stem melts and less termination.

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

LESS TERMINATION

The DNA sequence coding for the tract of U's is far away from the stem. It will not have even been transcribed when the secondary structure of the stem and loop causes pausing in the polymerase active site. Therefore there will be less termination.

(also okay) Effective termination requires a stem and loop structure to cause pausing in the RNA polymerase active site and a U-rich tract creating a relatively unstable RNA-DNA duplex as the newly synthesized sequence in the active site. If it is moved 100 nucleotides away the random sequence inserted will not cause efficient termination and there will be less termination.

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.

(This was mentioned in your notes and in lecture)

If a large number of unknown DNA fragments is fused to the DNA binding domain, then the large number of cDNAs encoding an activation domain will each activate transcription of the reporter or selectable marker used in the 2-hybrid screen. Since they do not actually bind to the test protein interaction domain they will not be bound in biochemical assays. It is for this reason that the candidate protein interaction domain is fused to the DNA binding domains and the large library of DNAs to be tested are fused to the activation domain. Even if they contain activation domains they cannot turn on the system unless they are brought to the DNA by protein:protein interaction with the test domain fused to the DNA binding domain.

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6. 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 planning the experiment that the student made?)

(This error is related to one of the controls shown in your notes and discussed in lecture)

In a chromatin immunoprecipitation assay there are two factors conferring specificity and leading to a positive signal. The antibody that immunoprecipitates the protein covalently linked to its DNA binding site and the PCR reaction using a pair oligonucleotides. The PCR reaction will amplify only those sequences between the oligonucleotide binding sites. If the fragments are only 600 nucleotides long and the steroid hormone receptor binding site is at -330 , it is very unlikely that a sequence in the middle of the gene coding for the mRNA will be immunoprecipitated with a bound receptor at -330 . Since the gene is likely to be several thousand nucleotides long with introns in it, this DNA is unlikely to be immunoprecipitated by an antibody to the steroid receptor bound to the site at -330 . By far the most likely error is that the student did not use PCR primers that lie on both sides of the region of the DNA, and the sequence to be amplified, was too far away from the binding site and was not precipitated, and therefore was not amplified in the PCR reaction.

(A common control in these assays is to show that a sequence on the same gene a considerable distance away is not immunoprecipitated and amplified, demonstrating that the reaction is really specific for a protein bound to the gene)