

Name KEY

Social Security Number _____

BIOCHEMISTRY 353, SPRING 2001 SECOND HOUR EXAM, MARCH 7, 2001

Before you start, PRINT your name and at least the last 4 digits of your social security number on the top of this 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 7 PAGES LONG. BEFORE STARTING CHECK TO BE SURE THAT YOU HAVE ALL OF THE PAGES.

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

Part I _____/30 points

Part II _____/24 points

Part III _____/20 points

Part IV _____/26 points

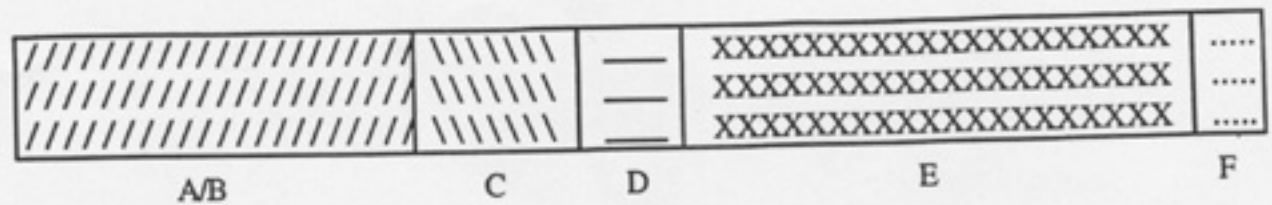
TOTAL _____/100 points

Part I. TRUE-FALSE (30 points, 3 points each) Circle T for statements which are True and F for statements which 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. RNA polymerase II is the least sensitive to α -amanitin of the eukaryotic RNA polymerases. RNA polymerase II is the MOST sensitive to α -amanitin.
- T (F) 2. Mutations which replaced three of the leucine residues in the leucine zipper region of a protein with glycine would probably have NO effect on the ability of two monomers of the protein to dimerize. These mutations would decrease interaction between hydrophobic side chains
- (T) F 3. For a constitutively expressed, non-regulated gene, mutations which change the sequence of the -10 region so that it is more distant (more different) from the -10 consensus sequence usually decrease the rate of prokaryotic transcription. Closer to consensus, higher transcription, more distant decreased transcription.
- (T) F 4. Most multi-protein complexes which assemble after binding of a regulatory protein to the DNA and result in activation of transcription of a gene transcribed by eukaryotic RNA polymerase II contain an enzymatic activity which acetylates histones. Chromatin changes are an important part of the process of eukaryotic transcription activation.
- T (F) 5. In prokaryotic DNA binding proteins which contain the helix-turn-helix motif, one helix binds to nucleotides which protrude into the minor groove of the DNA, and the second strongly acidic helix stabilizes binding to the DNA by interacting with the phosphate backbone of the DNA. False several ways: second helix doesn't bind in the minor groove, is not strongly acidic & does not bind the phosphate backbone
- T (F) 6. Histone H1 is highly conserved in all organisms from yeast to humans because it is the first protein assembled into the histone octamer in the core nucleosome. False: H1 is not highly conserved, is Not found in yeast & is Not in the core histone octamer
- (T) F 7. Sigma factor is important for the correct initiation of transcription by prokaryotic RNA polymerase, but sigma factor is not required for elongation of previously initiated RNA chains. Sigma factor dissociates from RNA polymerase to allow elongation to proceed & the polymerase to exit the promoter region.
- (T) F 8. The presence of near consensus sequences for any two of these three elements, the TATA box, the initiator, or the downstream promoter element (DPE), is usually sufficient to define a start site for a eukaryotic RNA polymerase II promoter. Few genes have all 3.
- T (F) 9. The length of the linker DNA between core nucleosomes remains constant between different species. The linker distance varies widely
The length of DNA wrapped around the
- (T) F 10. In a chromatin immunoprecipitation (CHIP) assay the DNA is chemically cross-linked to proteins bound to the DNA primarily to prevent the proteins from falling off the DNA during the immunoprecipitation and washes. The DNA & protein must remain associated until the complex is physically isolated.

Part II. (24 points)

1. (12 points 4 points each) A schematic diagram (not drawn to scale) of the domain structure of a typical member of the steroid hormone receptor gene family is shown below. For each of the indicated domains describe one function usually associated with the domain.



Any ONE is accepted

- E domain Ligand binding, hormone binding, dimerization, transcription activation, nuclear localization (phosphorylation allowed but not really a function)
- C Domain DNA binding, DNA sequence recognition, binding hormone response element (HRE), nuclear localization, dimerization
- A/B domain (N-terminal domain) Transcription activation, transactivation, Phosphorylation (accepted, but not really a function)

2. (12 points, 3 points each) Use the steroid hormone receptor diagram shown above. You transfect into cells which do not contain their own steroid receptor, DNA constructs coding for shortened receptors containing the various steroid hormone receptor domains listed. Next to each domain write ONE letter corresponding to the relative reporter gene activity you expect if the truncated receptor is transfected into cells along with the correct hormone ligand for the receptor. (There may be more than one correct answer, if so, any ONE of the correct answers is sufficient. (The mutant receptor CONTAINS only the domains listed.)

- A. No activity
- B. Low activity
- C. Moderate activity
- D. High activity (about the same as the wild-type full length receptor)

- A or B 1. C domain No activation functions, No or very low activity
- A 2. A/B domain No DNA binding, no activity
- A 3. D,E, and F domains No DNA binding, no activity
- C, B, D 4. C,D,E, and F domains Moderate or low activity most common, rarely high activity

Part III. Short Answer (20 points, 4 points each) 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 which is incorrect, some credit will be deducted.

1. The wild type lac repressor and a mutant lac repressor which is unable to bind the inducer are expressed in the same bacteria. High level expression of the lac operon genes is never seen in these cells, even in the presence of inducer.

The lac repressor mutant binds to the operators and does not come off, because it does not bind to the inducer. if the wild-type lac repressor is present, as soon as it binds inducer and dissociates from the operators, the mutant repressor will bind, blocking transcription

(not required: This is a dominant negative mutant. When the dominant negative mutant is expressed in the same cells as the wild-type protein, the mutant phenotype predominates and is dominant.)

2. The protein SRY (sex reversal Y chromosome) is the master switch for mammalian sex determination. Insertion of the DNA sequence coding for SRY into an otherwise normal female mouse embryo is sufficient to cause it to become a nearly normal looking male mouse. SRY is a sequence-specific DNA binding protein. SRY mutants which have lost the ability to bind DNA do not function. However in transient transfection experiments in which several copies of the SRY binding site are linked to a test promoter and reporter and SRY is expressed in the cells, there is no SRY-dependent activation of transcription of the reporter gene. Similarly, no SRY-dependent transcription is observed in cell-free transcription experiments when purified SRY is used to activate a test gene containing several copies of the SRY DNA binding site. Based on the contents of this course, what is ONE way in which SRY could work?

Either one of these answers is acceptable

SRY could work by bending the DNA, thereby bringing other proteins bound to the other DNA binding sites in its target genes into proximity. since SRY only bends DNA, it does not activate transcription when bound to a synthetic construct containing only its DNA binding sequence

(this is its mechanism of action, and was mentioned in lecture)

also acceptable: SRY could work by forming heterodimers which stabilize binding of other transcription regulatory proteins, (which contain activation functions), to their recognition sequences on DNA. since the synthetic construction does not contain the binding sites for these activators, it would show no activity.

3. The enzyme β -galactosidase can convert a colorless substrate to a blue product. The enzyme luciferase can produce light by breaking down ATP. The genes coding for these two proteins are widely used as reporter genes in transient transfection assays of mammalian cells. What are TWO properties (2 points each) possessed by the β -galactosidase and luciferase systems which make them good reporter genes in transfections?

Any two of these several answers is acceptable:

- There are sensitive assays for detection of low levels of these proteins.**
- The assays for these proteins are simple and rapid.**
- These enzymes are not present in most vertebrate cells, so there is a very low assay background.**
- The enzyme activity and stability of these proteins is NOT changed in the presence of regulatory proteins. Protein activity is therefore proportional to the rate of transcription of their genes**
- The processing and/or cytoplasmic mRNA stability and/or translational efficiency of these mRNAs is not regulatory by hormones or regulatory proteins. The enzyme activity of the proteins is proportional to the amount of their mRNAs, which is proportional to the rates at which their genes are transcribed. (only one of these sites need be mentioned, processing/splicing, mRNA stability/degradation, or translation control)**

4. Because eukaryotic cell-free transcription reactions contain a great many proteins, a first year graduate student was worried that the TATA box binding protein (TBP) in the reaction might become degraded. To determine how much TATA box binding protein was in the reaction, the student added an excess of a polyclonal antibody to TATA box binding protein prior to the start of the reaction. (Note: A polyclonal antibody is a mixture of individual antibodies raised against all of the different antigenic determinants on a protein.) Why were there no specific transcripts from the promoter.

Either of these answers is acceptable

The antibodies to the TATA binding protein were added before the protein had a chance to bind to the TATA box. These antibodies will bind to the several potential antibody binding sites on the protein, occupy sites needed for TBP to bind to the TATA box and prevent TBP from binding to the DNA.

(remember that a similar sequestration mechanism carried out by a TBP binding protein in the basal transcription apparatus regulates TBP activity)

Also acceptable: Binding of the antibodies to their antigenic determinants on TBP blocks sites of protein:protein interaction between TBP and other components of the basal transcription apparatus, thereby preventing formation of a transcription complex at the start site.

5. In early *Drosophila* Embryos the cells divide very rapidly. To identify proteins important in this process, you immobilize Histone H3:H4 dimers on an affinity column. In extracts from *Drosophila* early embryos you find that a substantial amount of a protein with the properties listed below binds to the histones on the affinity column. (i) the protein binds to all of the histones except histone H1. (ii) The protein does not carry out any enzyme reaction in DNA replication. (iii) The protein is strongly negatively charged. (iv) The protein is much more abundant in cells carrying out rapid DNA synthesis than in cells which are not replicating their DNA. What is the most likely function of this protein?

The protein is most likely involved in the pre-assembly of nucleosome core particles. Thus, the protein will bind all of the histones, except for H1, which is not in the core particle. Since fewer nucleosomes need to be laid down, the level of the protein would be expected to be lower in proteins now carrying out rapid synthesis of new DNA. Since histones interact with the negatively charged phosphate backbone of DNA, the protein would be expected to carry a negative charge to simulate a phosphate backbone on DNA

(not required: proteins with these properties do exist in rapidly dividing cells.)

(DO not write below this point)

Part IV (24 points) Matching (3 points each; there is NO PENALTY FOR GUESSING)

Next to each of the statements write the **NUMBER** of the item on the list which best carries out this reaction or process, or completes the phrase. If none of the items in the list is an appropriate answer, write in 10. You may use a given answer more than once, or not at all.

1. CTD, C terminal domain of RNA polymerase II
2. Histone deacetylase (HDAC)
3. RNA polymerase I
4. RNA polymerase III
5. Enhancer
6. Micrococcal Nuclease
7. Histone H3
8. Magnesium
9. TBP (TATA box binding protein)
10. None of the above

- 4 a. Carries out the transcription of genes encoding 5S RNA. RNA polymerase III
- 5 b. Activates transcription in a largely position-independent and orientation independent fashion. defines an enhancer
- 10 c. A histone not found in the core nucleosome Histone H1, not listed
H3 is in the core nucleosome
- 1 d. A short repeated amino ^{acid} sequence which is essential for transcription and is thought to become phosphorylated. Many copies, phosphorylation, may help RNA polymerase II initiate & begin elongating, CTD
- 10 e. This metal is coordinated by multiple cysteine or cysteine and histidine residues in the "finger" structures of many eukaryotic DNA binding transcription factors. Zinc
Zinc Finger, Not listed
- 2, 7 f. Often found in complexes which repress transcription Histone deacetylase is the appropriate answer. Since H3 & other core histones are associated with repressed DNA, I will accept 7 also
- 6 g. Cuts chromatin, and at low concentrations preferentially digests the linker region between nucleosomes. Used to define linker and core DNA
- 1 h. Binds one of the proteins involved in adding the 5'-cap (m⁷-Gppp) to newly synthesized mRNA transcripts. CTD couples transcription and processing
its ability to bind the guanyl transferase important in 5' Cap addition
This was mentioned in the lecture & is in Lewin's book