

Name _____

BIOCHEMISTRY 353, SPRING 2003 SECOND HOUR EXAM, MARCH 12, 2003

Before you start, PRINT your name in the space provided 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. However, you **MUST** indicate in the space devoted to that questions answer that the answer is on the **BACK** of the page or it will not be graded.

THIS EXAM IS X 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 _____/20 points

Part III _____/27 points

Part IV _____/23 points

TOTAL _____/100 points

Part I. TRUE-FALSE (30 points, 3 points each) Circle **T** for statements that are True and **F** for statements that are false. There is no deduction for wrong answers. You are free to guess.

- T F 1. When the DNAs of humans and the cold blooded amphibian *Xenopus laevis* are compared, more highly conserved sequences usually represent functionally important exons and regulatory regions and less highly conserved regions generally represent non-coding DNA.
- T F 2. Deacetylation of histone tails usually reduces expression of genes to which the deacetylated histones are bound.
- T F 3. The ribose sugars at the 5' and 3' ends of cytoplasmic eukaryotic mRNAs contain free 3'-OH groups.
- T F 4. Transcription of pre-mRNAs by RNA polymerase II terminates when the polymerase reaches a stretch of about 200 Ts in the DNA and the poly A tail is transcribed.
- T F 5. Naturally occurring and artificially evolved ribozymes generally have a lower turnover number (maximum reaction speed) than protein enzymes.
- T F 6. RNA molecules are often used in *in vitro* genetic selection because they can be single-stranded, or partially double stranded, and are more flexible, and are thought to assume more diverse shapes than double-stranded DNA.
- T F 7. Coactivators do not themselves bind to DNA, but instead serve as bridging proteins that link DNA sequence specific activators to other mediator proteins, or to the basal transcription apparatus.
- T F 8. Binding of a steroid hormone to a steroid receptor causes zinc to bind to the aspartate residues in the zinc fingers.
- T F 9. The first step in the degradation of most eukaryotic mRNAs is the enzymatic removal of the 5'-cap (ie. decapping the mRNA).
- T F 10. If a eukaryotic organism has no DNA sequence coding for guide RNAs, it cannot carry out A → I RNA editing.

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Part II. Fill in. (2 points each)

In the space provided fill-in the word, or words, that best completes the statement. There may be more than one correct answer. Only the first written answer will be graded.

a. _____ is a histone not present in the core nucleosome octamer.

b. Steroid hormone receptors bind to their recognition sequences on DNA using the _____ DNA binding motif.

c. In RNA editing, the nucleotide most often inserted into an RNA is _____.

d. _____ binds to sequences near the 3' end of cytoplasmic eukaryotic mRNAs.

e. Replacing the _____ of glucocorticoid receptor with the same segment of the estrogen receptor will cause the glucocorticoid receptor to bind to the DNA sequence normally recognized by the estrogen receptor.

f. When _____ binds to the estrogen receptor, helix 12 of the estrogen receptor assumes a conformation in which it occupies the binding site normally occupied by coactivators.

g. _____ is a protein in the RNA polymerase II basal transcription apparatus that directly binds to a DNA sequence.

h. Proteins with histone acetylase (HAT) activity acetylate the amino acid _____ in histone tails.

i. This is one (any one is okay) _____ of the three elements that help define start sites for RNA polymerase II transcription.

j. In RNA interference (RNAi), _____ cleaves long double stranded RNAs into short 21-23 nucleotide double stranded RNAs.

Part III. Matching (27 points, 3 points each). There is no penalty for guessing)

Nucleic acids and their interactions with proteins play an important role in biochemistry. Next to each of the statements describing a reaction or process, write the **NUMBER** of the nucleic acid, sequence, type of nucleic acid, nucleic acid structure from the list below that is important in an interaction or process, or describes the function of the nucleic acid. If none of the specific items in the list is appropriate for that statement, fill in 1.

- a. _____ Found at the 3'-end of nearly all eukaryotic mRNAs.
 - b. _____ Found in the cap structure at the 5'-end of most eukaryotic mRNAs.
 - c. _____ Stimulates the degradation of mRNAs containing a sequence complementary to it.
 - d. _____ The conserved sequences at the ends of an intron.
 - e. _____ A component of the TFIID complex binds to this sequence
 - f. _____ Binds to the CTD repeats in RNA polymerase II
 - g. _____ The half site sequence bound by many steroid/nuclear receptors.
 - h. _____ The unusual electrophoretic mobility of this structure led to the discovery of its role in splicing of mRNA precursors.
 - i. _____ Binds a metal ion required for splicing of mRNA precursors
1. None of the items below corresponds to the statement
 2. U6 snRNA
 3. 5'-aGGTCA-3'
 4. 5'-AAUAAA-3'
 5. poly(A)
 6. A 5'-ppp-3' triphosphate linkage
 7. 5SRNA
 8. 5'-GU----(N)_n-----AG-3'
 9. lariat structure
 10. 21-23 nucleotide double stranded RNA

- 11. TATAA
- 12. tRNA

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Part III. (23 points) Long Answer

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 may be sufficient. If you keep writing and write something that is incorrect, some credit will be deducted.

(4 points each, unless otherwise noted) Provide a brief biochemical explanation for each of the following statements or observations. Your answers should reflect the contents of this course.

1. A biotechnology seeking to find new targets for killing the *Trypanosome* that causes sleeping sickness decides to focus on inhibitors of RNA ligase. Why?

2. A student attempting to use *in vitro genetic* selection (SELEX) to select an RNA that will bind caffeine use synthesis with all 4 nucleotides present at each position to create a very large pool of candidate DNAs with random sequences. These sequences are transcribed into RNAs containing only random sequence and the pool of RNAs containing random sequence is bound to immobilized caffeine. The bound RNAs were recovered from the immobilized caffeine and RT-PCR using specific primers was used to copy the RNA back into DNA. Although the student is positive that some RNAs bound to the sequence and were recovered, after the RT-PCR there is no DNA. What did the student do wrong?

3. The enzyme poly(A) ribonuclease catalyzes progressive removal of As from the end of an mRNA and is part of the PARN complex. In an effort to learn more about the function of this enzyme, the student used RNA interference (RNAi) to “knockdown” the level of poly(A) ribonuclease. The knockdown was successful and the enzyme almost completely disappeared from the cells. Before they died, the cells in which poly(A) ribonuclease was knocked down showed an increase in the overall rate of protein synthesis. Explain this observation?

4. The estrogen receptor is essential for reproduction, but is not essential for cells in culture. Most cultured cells do not contain estrogen receptor. In activating transcription estrogen receptor recruits coactivator proteins that in turn recruit the large protein CBP or its close relative p300. To further analyze the role of CBP/p300 in estrogen receptor mediated transcription a student used RNA interference to knockdown the level of CBP/p300. the knockdown was successful and the cells promptly died. Why?

5. “Wake-up” is a (fictitious, made-up) gene that causes students to get up early in the morning and come to early morning classes. The Wake-up gene in students who fail to get up in time for early morning lectures was isolated and analyzed.