

Name _____ KEY _____

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 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 _____/20 points

Part III _____/27 points

Part IV _____/23 points

TOTAL _____ **KEY** _____/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** 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 usually represent non-coding DNA.
SELECTION AGAINST CHANGES IN CODING AND REG. REGIONS
- T** 2. Deacetylation of histone tails usually reduces expression of genes to which the deacetylated histones are bound.
MORE POSITIVE CHARGE, TIGHTER BINDING TO DNA, BETTER REPRESSION
- T** 3. The ribose sugars at the 5' and 3' ends of cytoplasmic eukaryotic mRNAs contain free 3'-OH groups.
5'-PPP-5' AT 5'- CAP LEAVES FREE 3'-OH
- F** 4. Transcription by RNA polymerase II terminates when the RNA polymerase reaches a stretch of about 200 Ts in the DNA and the poly A tail is transcribed.
POLY(A) IS ADDED TO RNA POST-TRANSCRIPTIONALLY
- T** 5. Naturally occurring and artificially evolved ribozymes generally have a lower turnover number (maximum reaction speed) than protein enzymes.
DIVERSE, BUT USUALLY VERY SLOW, MENTIONED IN CLASS
- T** 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** 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.
- F** 8. Binding of a steroid hormone to a steroid receptor causes zinc to bind to the aspartate residues in the zinc fingers.
ZN FINGERS BIND CYSTEINE, LIGAND DOES NOT STIM. BINDING
- F** 9. The first step in the degradation of most eukaryotic mRNAs is the enzymatic removal of the 5'-cap (ie. decapping the mRNA).
FIRST STEP IS DEADENYLATION
- F** 10. If a eukaryotic organism has no DNA sequence coding for guide RNAs, it cannot carry out A→I RNA editing.
A→I EDITING USES ENZYMES NOT A GUIDE SEQUENCE, INSERTION OF U USES GUIDE

i. The **TATA BOX, or INITIATOR, or DOWNSTREAM PROMOTER ELEMENT (DPE)** 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), **DICER, or RNase III** cleaves long double-stranded RNAs into short 21-23 nucleotide double-stranded RNAs.

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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, or 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. 5 Found at the 3'-end of nearly all eukaryotic mRNAs.
- b. 1 Found in the cap structure at the 5'-end of most eukaryotic mRNAs.
5'-PPP-5' is found in the cap not 5'-PPP-3'
- c. 10 Stimulates the degradation of mRNAs containing a sequence complementary to it.
- d. 8 The conserved sequences at the ends of an intron.
- e. 11 A component of the TFIID complex binds to this sequence
- f. 1 Binds to the C-Terminal Domain (CTD) repeats in RNA polymerase II
- g. 3 Palindromes or direct repeats of this half-site sequence are bound by some steroid/nuclear receptors.
- h. 9 The unusual electrophoretic mobility of this structure led to the discovery of its role in splicing of mRNA precursors.
- i. 2 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 company seeking to find new targets for preventing the severe disease called sleeping sickness, which is caused by a *Trypanosome*, decides to focus on inhibitors of the *Trypanosome's* RNA ligase. Why?

Answer: *Trypanosomes* make extensive use of RNA editing involving the insertion of Us and removal of nucleotides to change the sequence of their mRNAs and the amino acid sequence of their proteins. (Not required: These continual changes in protein sequence help them evade the host immune system). After insertion of U or removal of nucleotides, the enzyme RNA ligase is required to covalently join the two ends of the mRNA that contain the added or deleted nucleotide(s). If RNA ligase is inhibited the mRNAs will be in pieces and functional proteins will not be produced. This should lead to death of the organism, or poor growth, or inability to evade the host immune system.

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

Answer: Since the sequence contains only random sequence and specific primer sequences were used to carry out the RT and PCR, they will be very unlikely to hybridize to the selected sequences and there will be no product produced in the RT-PCR reactions. (Not required: This is why pools used for *in vitro* genetic selection normally contain fixed sites that can serve as sites for hybridization by specific primers. Random sequence is between these fixed sites so that the random sequence can be amplified using specific primers.)

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3.The enzyme poly(A) ribonuclease catalyzes progressive removal of As from one 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 mRNA and protein. 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?

Answer: Eliminating this protein will block the major pathway for eukaryotic mRNA degradation. This pathway begins with progressive digestion of the 3’poly(A) tail. Since these mRNAs will not be degraded and initially they will continue to be synthesized, their levels will increase in cells. Since the mRNAs remain functional, the increased levels of so many mRNAs will produce an overall increase in the amount of protein synthesis. (Not required: Altered levels of so many mRNAs and proteins will ultimately be lethal and result in death of the cells)

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?

Answer: Since the estrogen receptor is not essential, it is unlikely that elimination of CBP kills the cells by blocking estrogen receptor mediating transcription. This data suggests that CBP/p300 has additional functions in cells beyond working with estrogen receptor. Since CBP/p300 is important in the action of cyclic AMP and many other transcription regulatory proteins, its loss would have a widespread effect on transcription and would likely result in cell death. (Not required: in class we discussed how the many regulatory pathways CBP/p300 are involved in means that it is limiting for any one pathway and these different pathways all compete for the available CBP/p300. Thought item: How could you show that CBP/p300 was limiting for transcription?)

5. A student isolated the RNA-protein complexes found in the nucleus. The student wanted to study only RNAs that were mRNAs destined to be exported from the nucleus to the cytosol, not partially spliced pre-mRNAs, or pieces of introns destined to be destroyed in the nucleus. Suggest one property or difference between mRNA-protein complexes destined for export and protein-RNA complexes containing either partially spliced pre-mRNAs, or pieces of introns, or one way the cell might distinguish between them.

Answer: Proteins that are associated with functional mRNAs, such as the CAP binding complex can be recognized by the cell transport system. This protein complex would not be associated with an excised intron, or fragment of an intron. Similarly, partially spliced RNAs will still have splicing factors associated with them and are recognized by the transport system as not-ready for transport.

Also acceptable: the Poly(A) binding protein bound to the poly(A) tail at the 3'-end of the mRNA will not be found on excised introns and can be used to help mark mRNAs for export.

6. (3 points) A student mutated amino acids in the clamp of RNA polymerase II so the clamp no longer swings over the active site on binding of substrates. What would be the likely effect on the length of the RNA transcripts? Briefly explain the reasoning behind your answer.

Answer: Shorter (1 point).

(2 points) The clamp helps to hold the template in the RNA polymerase II active site. If the clamp does not swing across the active site, the DNA template will be much more likely to dissociate away from the enzyme. The RNA:DNA complex can dissociate in solution while the displaced DNA strand reanneals. This will cause premature termination of transcription, resulting in the production of shorter transcripts.