

Name _____ KEY _____

BIOCHEMISTRY 353, SPRING 2003, FINAL EXAM, MAY, 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 SECTION OF THE FINAL 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

Section I

Part I _____ KEY _____ / 20 points

Part II _____ KEY _____ / 20 points

Part III _____ KEY _____ / 28 points

TOTAL _____ KEY _____ / 68 points

Part I. TRUE-FALSE (20 points, 2 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. The second step in the degradation of most eukaryotic mRNAs is the enzymatic removal of the 5'-cap ("decapping").
DEADENYLATION, FOLLOWED BY DECAPPING FOLLOWED BY 5'→3' DEGRADATION IN YEAST AND 3'→5' DEGRADATION IN VERTEBRATES
- F** 2. Chromatin immunoprecipitation and other methods demonstrate that steroid hormone receptors are tightly bound to their correct DNA response elements near the promoter regions of steroid hormone regulated genes, even when no hormone is present.
CHIP ASSAYS SHOW THAT STEROID RECEPTORS ARE NOT USUALLY BOUND TO THEIR TARGET SITES UNTIL AFTER THEY BIND HORMONE
- T** 3. Adenosine deaminases are important in RNA editing and targeted disruption of these adenosine deaminases is lethal in knockout mice.
- T** 4. Acetylation of histone tails usually increases transcription of genes to which the acetylated histones are bound.
ACETYLATION REDUCES THE EFFECTIVE CHARGE OF THE HISTONES DECREASING THEIR INTERACTION WITH DNA AND INCREASING TRANSCRIPTION WHILE DEACETYLATION INCREASES CHARGE AND REPRESSES TRANSCRIPTION
- T** 5. The intron nearest to the 5'-end of a pre-mRNA transcript is the intron most likely to be removed first.
SPLICING OFTEN STARTS DURING TRANSCRIPTION AND THE MOST 5' INTRON IS MOST OFTEN THE FIRST INTRON TO BE REMOVED
- T** 6. key step in demonstrating that DNA is the hereditary material was the demonstration that heat-treated bacteria could transfer a factor to live non-pathogenic bacteria and make them pathogenic.
THE HEAT-KILLED BACTERIA WERE NOT INFECTIOUS AND THEIR PROTEINS WERE DENATURED, BUT THEIR DNA LARGELY SURVIVED AND COULD BE TRANSFERRED
- T** 7. If you very rapidly label the newly synthesized DNA in an *E. coli* mutant containing 500% of the normal level of DNA ligase, the fragments of newly synthesized DNA (i.e. "Okazaki fragments") isolated from the bacteria containing the increased DNA ligase activity will be longer than those in *E. coli* containing normal DNA ligase levels.
THE ADDITIONAL DNA LIGASE WILL LEAD TO FASTER LIGATION AND THEREFORE TO LONGER DNA FRAGMENTS
- F** 8. *E. coli* RNA polymerase does not copy DNA because it cannot bind to and recognize nucleic acids containing deoxyribose.
E. COLI RNA POLYMERASE BINDS DNA AT PROMOTERS AND ALSO THE LENGTH OF THE DNA IN ORDER TO PRODUCE RNA

T

9. An RNA:DNA duplex is formed in nucleic acid synthesis by reverse transcriptase (RNA-dependent DNA polymerase).

REVERSE TRANSCRIPTASE USED A DNA PRIMER (LEADING TO AN RNA DNA DUPLEX) TO SYNTHESIZE A COMPLEMENTARY DNA FROM AN RNA TEMPLATE.

F

10. If the helix-turn-helix region of the Cro repressor were used to replace the helix turn-helix region of the cyclic AMP binding protein (CAP, CRP), transcription of the Cro genes would probably be decreased in the presence of cyclic AMP.

CAP/CRP IS A TRANSCRIPTION ACTIVATOR THAT BINDS TO DNA MORE TIGHTLY WHEN CYCLIC AMP IS PRESENT AND WILL THEREFORE INCREASE TRANSCRIPTION WHEN CYCLIC AMP IS PRESENT

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Part II. Fill in. (2 points each, 20 points total)

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. RNA polymerase **II** (I, II or III) is the most sensitive to inhibition by α -amanitin of the three eukaryotic RNA polymerases.
- b. Histone **H1** is not highly conserved and is not found in yeast. (Think. Based on what you know about the various histones you should be able to deduce the answer even if you do not know it.)
- c. In RNA editing, the nucleotide produced by the adenosine deaminase reaction is **INOSINE, I**.
- d. (1 point each) Two of the three elements important in specifying a start site for a eukaryotic RNA polymerase II are **TATA BOX** and **INITIATOR**. **OR DOWNSTREAM PROMOTER ELEMENT, DPE**
- e. The unusual electrophoretic mobility of the **LARIAT STRUCTURE** led to the discovery of its role in the splicing of mRNA precursors.
- f. Although the proteins bind to completely different nucleic acid sequences, the DNA binding sites for lac repressor and steroid hormone receptors are both **PALINDROMES (ALSO OKAY, IMPERFECT PALINDROMES; SYMMETRICAL)**.
- g. One of the strands of DNA has the sequence 5'-G T C A T G T-3'. An **RNA** transcribed from the strand of DNA **complementary** to this sequence will have the sequence **5'-G U C A U G U-3'** (NO T IN RNA).
- h. **LUCIFERASE, B-GALACTOSIDASE, CAT; CHLORAMPHENICOL ACETYL TRANSFERASE (any one is okay)** is ONE enzyme widely used as a reporter gene in transient transfection experiments that is not normally present in human cells.
- i. DNA polymerase III carries out synthesis of DNA. In addition to its ability to polymerize DNA, one additional enzymatic activity that can be carried out by DNA polymerase III is **5'→3' exonuclease or 3'→5' exonuclease (either one is okay)**.

j. In dideoxy DNA sequencing, carbon **3** (write in the number) on the deoxyribose sugar contains a hydrogen (H) instead of its normal OH group.
(THE ABSENCE OF THE 3'OH RESULTS IN TERMINATION OF THE GROWING CHAIN)

Part III. (28 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. The DNA of a newly discovered disease-causing virus has the following base composition: A=21%; G=32%; T=30%; C=17%. What important fact does this tell you about the organisms DNA?

FOR DOUBLE-STRANDED DNA A=T, G=C AND A+G=T+C. (NOT REQUIRED: THESE ARE SOMETIMES CALLED CHARGAFF'S RULES). THE BASE COMPOSITION OF THIS DNA DOES NOT FOLLOW THE RULES OF BASE PAIRING FOR DOUBLE-STRANDED DNA. THIS TELLS US IS THAT THE VIRUS MOST LIKELY CONTAINS SINGLE-STRANDED DNA.

2. The amino acid sequences of the human estrogen receptor DNA binding domain and the estrogen receptor DNA binding domain in the cold-blooded amphibian, *Xenopus laevis*, differ by only one out of 88 amino acids. A student made an siRNA from a stretch of DNA in which the amino acids sequences of the human and *Xenopus* DNA binding domains were identical. When the siRNA RNA was transfected into cultured human cells, human estrogen receptor mRNA and protein were knocked down. When the siRNA was transfected into *Xenopus* cells, the level of *Xenopus* estrogen receptor mRNA and protein was unchanged. Control experiments demonstrated that after transfection, similar amounts of the siRNA were present in the human and *Xenopus* cells. Why was the attempt to knockdown of the *Xenopus* estrogen receptor unsuccessful?

THE siRNA WAS EFFECTIVELY TRANSFECTED INTO THE XENOPUS CELLS AND RNA INTERFERENCE WORKS IN A WIDE VARIETY OF EUKARYOTES. THEREFORE, THE MOST LIKELY EXPLANATION IS THAT THE NUCLEOTIDE SEQUENCES OF THE siRNA AND THE XENOPUS ESTROGEN RECEPTOR DNA BINDING DOMAIN WERE NOT IDENTICAL. ALTHOUGH THE PROTEIN SEQUENCES ARE HIGHLY CONSERVED, THE CONSIDERABLE EVOLUTIONARY DISTANCE BETWEEN XENOPUS AND HUMAN MEANS THAT THE THIRD NUCLEOTIDES, THE WOBBLE BASES, ARE FREE TO DIVERGE. MOST AMINO ACIDS ARE ENCODED BY MORE THAN ONE TRIPLET. THEREFORE THE siRNA COMPLEMENTARY TO THE HUMAN mRNA SEQUENCE WAS MISMATCHED WITH THE XENOPUS mRNA SEQUENCE, THERE WAS NOT EFFICIENT HYBRIDIZATION AND THE RNA INTERFERENCE SYSTEM WAS NOT ACTIVATED.

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3. In order to properly turn on and off transcription of their specific target genes steroid hormone receptors must carry out multiple functions or biological activities. These activities are often carried out by different domains or regions of the receptors. What are **two** functions steroid hormone receptors must be able to carry out? (Do not write turn on and off transcription. You need to write two functions that steroid receptors must carry out in order to turn transcription on and off).

(ANY TWO, 2 POINTS EACH)

BIND A SPECIFIC HORMONE LIGAND

BIND A SPECIFIC DNA SEQUENCE OR BIND TO A SPECIFIC HORMONE RESPONSE ELEMENT

BIND TO COACTIVATORS OR COREPRESSORS

ALSO ACCEPTABLE: BIND TO HEAT SHOCK PROEINS AND CHAPERONES

4. What are **two** reasons or properties that make double stranded DNA a better material than single-stranded RNA for the storage of genetic information. (Don't just say its double stranded as one reason. If you write about this you must explain why it's better.)

THE DOUBLE-STRANDED STRUCTURE PROVIDES TWO COMPLEMENTARY STRANDS AND THIS ALLOW DAMAGE IN ONE STRAND TO BE REPAIRED BY USING THE SEQENCE INFORMATION IN THE SECOND STRAND.

DEOXYRIBOSE LACKS A HYDROXYL GROUP AT C-2. IN RIBOSE, THIS HYDROXYL GROUP CAN ACT AS A NUCLEOPHILE AND CATALYZE BREAKAGE OF THE 3'-5' PHOSPHODIESTER BOND. (FOR EXAMPLE, RNA IS MUCH MORE LABILE AT BASIC pH THAN DNA)

DNA CONTAINS T AND RNA CONTAINS U. DEAMINATION IS A COMMON FORM OF DAMAGE TO NUCLEIC ACIDS. DEAMINATION OF T RESULTS IN THE ABNORMAL BASE METHYL-C. THIS MAKES RECOGNIZING THIS DAMAGE EASIER. DEAMINATION OF U GENERATES C.

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5. (12 points) “Wake-up” is a (fictitious, made-up, not real) gene that causes students to get up early in the morning and come to early Monday morning classes. The mRNA coding for the “wake-up” protein three students, who are members of the same family, and do not get up in time for early Monday morning lectures, was isolated and the nucleotide sequence was determined and compared to the sequence of the “wake-up” mRNA in several unrelated control students who come to early Monday morning classes. The DNA sequence of the “wake-up” gene in the three family members who do not come to early Monday morning lectures and in the control students who do come to Monday morning lectures was also determined and compared.

5. The “wake-up” mRNA of the 3 students who do NOT get up for early Monday morning lectures was missing 152 internal nucleotides when it was compared to the “wake-up” mRNA of the control students who do attend Monday morning lectures. The deletion of the 152 nucleotides was near the middle of the coding region of the mRNA. The DNA sequences of the “wake-up” genes in the 3 family members who do not get up for early Monday morning lectures and the control group who do get-up for lectures was different by only one nucleotide. No nucleotides were deleted and the mutation was in an intron.

A. (5 points) Briefly describe or diagram (at the molecular level) a mechanism through which this single nucleotide change could cause the INTERNAL deletion seen in the mRNA.

(3 POINTS) THE MOST LIKELY WAY IN WHICH A SINGLE NUCLEOTIDE CHANGE IN AN INTRO COULD CAUSE A 152 NUCLEOTIDE DELETION IN THE SPLICED mRNA IS IF THE MUTATION ALTERED SPLICING SO THAT AN EXON THAT WAS NORMALLY PRESENT IN THE mRNA WAS SKIPPED.

(2 POINTS) FOR EXAMPLE, IF EXON TWO OF THE GENE CONTAINED 153 NUCLEOTIDES AND THE NORMAL SPLICING PATTERN IS THAT THE INTRON BETWEEN EXONS 1 AND 2 IS REMOVED BY SPLICING, AND THE INTRON BETWEEN EXONS TWO AND THREE IS REMOVED BY SPLICING, THEN ALL OF THE EXONS WILL BE PRESENT INCLUDING THE 152 NUCLEOTIDE LONG EXON 2. IF THE MUTATION CHANGED THE 3' SPICE SITE IN THE INTRON PRECEEDING EXON 2 SO THAT IT WILL NOT BE RECOGNIZED (FOR EXAMPLE BY MUTATING ONE NUCLEOTIDE IN THE CONSERVED AG SEQUENCE AT THE INTRON-EXON BOUNDARY), THEN THE PLICING MACHINERY WILL SKIP OVER THIS MUTANT SPICE JUNCTION AND USE THE NEXT AVAILABLE SPICE JUNCTION RESULTING IN THE LOSS OF THE 152 NUCLEOTIDE EXON 2.

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- B. (4 points) What would happen to the “wake-up” protein translated from the mRNA containing the 152-nucleotide deletion near the middle of the CODING region of the mRNA.

(Hint: Think about what happens when you have a mutant that contains a deletion of 152 nucleotides in the middle of the coding region of an mRNA)

SINCE EACH CODON IS THREE AMINO ACIDS, THE DELETION OF 152 NUCLEOTIDES DOES NOT RESULT IN THE LOSS OF AN INTEGRAL NUMBER OF CODONS. SINCE THERE IS NO INTERNAL PUNCTUATION IN THE GENETIC CODE, ALL OF THE PROTEIN PAST THE DELETION WILL BE OUT OF READING FRAME AND MAY CONTAIN TERMINATION CODONS. THE PROTEIN WILL ALSO BE MISSING ABOUT 50 AMINO ACIDS, AND THIS MAY ALTER ITS STRUCTURE AND/OR CAUSE ITS RAPID DEGRADATION.

- C. (3 points) Briefly describe or diagram the difference between the “wake-up” protein in the family members who do not get up early and attend Monday morning lectures and the control students who do attend the lectures.

**THE MUTANT PROTEIN WILL BE SHORTER THAN THE NORMAL PROTEIN BY AT LEAST 50 AMINO ACIDS THAT ARE CODED BY THE DELETION. THE MUTANT PROTEIN WILL MAYBE FURTHER SHORTENED BY PREMATURE TERMINATION RESULTING FROM THE PRESENCE OF TERMINATION CODONS WHEN THE MUTANT mRNA IS TRANSLATED OUT OF THE CORRECT READING FRAME. ALL OF THE AMINO ACIDS TO THE 3' SIDE OF THE DELETION WILL BE DIFFERENT DUE TO THE SHIFT OF READING FRAME AND THE RESULTING FRAMESHIFT MUTATION WILL CONTAIN A COMPLETELY DIFFERENT 3' AMINO ACID SEQUENCE THAN THE WILD-TYPE PROTEIN.
(EITHER OF THE LAST TWO STATEMENTS IS ACCEPTABLE, IE. TRUNCATION, OR FRAMESHIFT, BOTH ARE NOT NEEDED)**