

Name _____ KEY _____

BIOCHEMISTRY 353, FINAL EXAM, SECTION 1, MAY, 2002

Before you start, **PRINT** your name top of this page and every 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 state clearly 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 SECTION OF THE EXAM IS 6 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 _____ KEY _____ /24 points

Part II _____ KEY _____ /24 points

Part III _____ KEY _____ /20 points

TOTAL FOR SECTION 1 _____ KEY _____ / 68 points

Part I. TRUE-FALSE (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.**

- F** 1. Activation of transcription by eukaryotic transcription factors bound to DNA is much less common than repression of gene transcription by eukaryotic transcription factors bound to DNA.
- F** 2. The DNA binding domains of steroid hormone receptors contain bound iron.
- T** 3. When *E. coli* are grown in a medium containing glucose but no lactose, lac repressor is almost always bound to the Lac operator.
- T** 4. If radioactive 2'3' dideoxy ATP is added to a dideoxy sequencing reaction, the appropriate enzymes and components are all present, and there is not premature termination, after the reaction, radioactive 2'3' adenosine will be the last nucleotide at the 3'-end of the DNA.
- T** 5. Histone H1 is not found in the core histone octamer.
- T** 6. For a constitutively expressed non-regulated prokaryotic gene, mutations that change the sequence of the -10 region so that it contains a higher proportion of G:C base pairs than the starting sequence will usually decrease the rate of transcription of that gene.
- F** 7. In transcription by eukaryotic RNA polymerase III the newly synthesized RNA chain grows in the 3'→5' direction.
- T** 8. Addition of a new inhibitor that blocks the enzymatic activity of most histone acetylases (also called histone acetyl transferases or HATs) to a cell-free transcription system using a test gene organized into a native chromatin structure would likely decrease the rate of transcription of the test gene.
- F** 9. Transcription by RNA polymerase II almost always terminates at the last nucleotide of the AAUAAA sequence that specifies polyadenylation.
- F** 10. An important step in demonstrating that DNA is the hereditary material was the demonstration that DNase had no effect on the ability of material isolated from heat killed *Pneumococcus* bacteria to infect and kill mice.
- F** 11. If you very rapidly label the newly synthesized DNA in an *E. coli* mutant containing 5 times more DNA ligase than normal *E. coli*, the fragments of newly synthesized DNA (hint: Okazaki fragments) isolated from the bacteria containing the increased level of DNA ligase will be shorter than those in the bacteria containing normal DNA ligase levels.
- F** 12. A mutation in eukaryotic RNA polymerase II that prevented the “clamp” from closing would probably result in the synthesis of RNA transcripts that are longer than usual.

Part II. Matching: (24 Points, 2 points each, There is no penalty for guessing) Next to each of the statements write the **NUMBER** of the item on the list that best carries out the reaction or process. If none of the items on the list is an appropriate answer write in 15. You may use a given answer more than once, or not at all.

1. Bacteriophage T4 DNA ligase
2. Zinc
3. Histone deacetylase (HDAC)
4. RNA polymerase III
5. Hammerhead ribozyme
6. Luciferase
7. DNA topoisomerase I
8. DNA polymerase
9. Sigma factor or sigma subunit
10. Leucine zipper
11. 5'-CCGGTC-3'
3'-GGCCAG-5'
12. Sigma factor (or sigma subunit)
13. Luciferase
14. Magnesium
15. None of the above

- ___ **8** ___ a. An enzyme that requires a primer to copy DNA.
- ___ **1** ___ b. An enzyme that covalently joins two blunt ended double stranded DNA fragments in the presence of ATP.
- ___ **15** ___ c. Recognizes the AAUAAA sequence near the 3'-end of most eukaryotic mRNAs.
- ___ **7** ___ d. An enzyme that increases the linking number of negatively supercoiled DNA by 1. In other words, it makes the linking number less negative.
- ___ **5** ___ e. Base pairs with a complementary sequence in its RNA substrate and cleaves the substrate.
- ___ **4** ___ f. This enzyme correctly initiates transcription even if all of the sequence upstream of the transcription start site is deleted.
- ___ **6** ___ g. This protein is widely used as a reporter gene in transient transfections of eukaryotic cells.
- ___ **3** ___ h. When this enzyme is inhibited by trichostatin A, the ability of histones bound to DNA to repress transcription is decreased.
- ___ **10** ___ i. A motif important in protein dimerization and in protein-protein interactions.
- ___ **9** ___ j. When this is missing from prokaryotic RNA polymerase transcription of nicked DNA increases.

___15___ k. The recognition sequence of the restriction enzyme EcoRI.

___14___ l. A metal ion important in the catalytic steps of RNA processing reactions and in the RNA polymerase reaction.

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 that is incorrect, some credit will be deducted.

1. The interferon genes do not contain introns. A student used PCR of genomic DNA to isolate an interferon clone that contains the entire genomic interferon gene of interest. Using this clone as a template, bacteriophage T7 RNA polymerase was used *in vitro* to produce an accurate RNA copy of the same strand of DNA that encodes the native cellular mRNA. When this *in vitro* synthesized RNA was microinjected into a *Xenopus* oocyte much less protein was produced than when the same amount of the cellular interferon mRNA was injected.

IN INTACT CELLS, EUKARYOTIC mRNAs ARE POSTTRANSCRIPTIONALLY MODIFIED BY THE ADDITION OF A 5'-CAP AND A 3'POLY(A) TRACT. SINCE THESE SEQUENCES ARE NOT ENCODED IN GENOMIC DNA, THEY WILL NOT BE PRESENT IN THE *IN VITRO* SYNTHESIZED RNA. THE 5' CAP AND POLY(A) TAIL ARE IMPORTANT FOR BOTH mRNA STABILITY AND FOR EFFICIENT TRANSLATION. SINCE THE INJECTED SYNTHETIC mRNA WILL BE RAPIDLY DEGRADED AND THE SMALL AMOUNT OF THE SYNTHETIC mRNA THAT REMAINS WILL BE TRANSLATED WITH LOW EFFICIENCY, MUCH LESS PROTEIN WILL BE PRODUCED THAN FROM AN EQUIVALENT AMOUNT OF THE CELLULAR mRNA

2. A student isolated a full-length cDNA clone of the sequence coding for the human oncogene called c-fos. When this clone was expressed in *E. coli*, very little c-fos protein was produced. C-fos is not very large and would be expected to fold correctly in bacteria. When the same clone was expressed in yeast, much more protein was produced.

THE GENETIC CODE IS DEGENERATE AND THERE ARE SEVERAL CODONS SPECIFYING MOST AMINO ACIDS. SOME CODONS ARE USED PRIMARILY IN BACTERIA AND OTHERS ARE USED PRIMARILY IN EUKARYOTIC CELLS. IF THE LEVEL OF tRNA FOR A SPECIFIC CODON THAT IS RARELY USED IN *E. COLI* IS LOW, THEN TRANSLATION WILL STALL AT THAT CODON AND THE PARTIALLY FOLDED C-FOS PROTEIN WILL BE DEGRADED. THIS WILL RESULT IN A LOWER YIELD OF PROTEIN THAN WAS SEEN IN THE EUKARYOTIC CELL. (THIS PROBLEM WAS MENTIONED AS ONE PROBLEM OF PROKARYOTIC EXPRESSION SYSTEMS.)

3. After isolating and purifying wild-type lac repressor a student attempted to reconstitute the regulation of lac operon transcription using only purified components. When the lac repressor, RNA polymerase and all 4 ribonucleoside triphosphates (rNTPs) were present, there was almost no transcription. Addition of the inducer IPTG (isopropyl thio galactoside) to the reaction containing the lac repressor and RNA polymerase as the only added proteins produced only a small increase in transcription. In contrast, when the student grew *E. coli* on glycerol and added IPTG, there was a very large increase in transcription of the lac operon.

THE COMPONENTS THE STUDENT ADDED WILL BE SUFFICIENT TO CAUSE LAC REPRESSOR TO COME OFF THE DNA. THIS IS NOT SUFFICIENT FOR EFFICIENT TRANSCRIPTION OF THE LAC OPERON. BINDING OF THE CATABOLITE GENE ACTIVATOR PROTEIN (CAP ALSO CALLED CYCLIC AMP RECEPTOR PROTEIN, CRP) IS REQUIRED FOR EFFICIENT TRANSCRIPTION OF THE LAC OPERON. CAP WILL BE PRESENT IN THE BACTERIA. SINCE THE E. COLI WERE NOT GROWN ON GLUCOSE, BUT ON GLYCEROL, THE CAP PROTEIN WILL BE ABLE TO BIND TO THE DNA AND IPTG WILL PRODUCE A LARGE INCREASE IN TRANSCRIPTION OF THE LAC OPERON.

4. The genome of the African clawed toad *Xenopus laevis* was duplicated millions of years ago and contains two sets of two copies of every gene (it is pseudotetraploid instead of diploid). A student isolated one cDNA clone for the serum protein *Xenopus laevis* albumin. To “knock-down” or eliminate albumin expression using RNAi, the student prepared a correct sized 23 nucleotide complementary RNA oligonucleotide. In control experiments the cDNA clone for *Xenopus* albumin either alone, or with the RNAi oligonucleotide, was cotransfected into *Xenopus* fibroblasts (which do not make any albumin on their own). Antibody studies indicated that cotransfection of the RNAi oligonucleotide into the cells completely blocked albumin production from the cDNA clone. When the RNAi oligonucleotide was transfected into *Xenopus* liver cells it only reduced their ability to make albumin by about 50%. Control experiments showed that the RNAi oligonucleotide was successfully transfected into all of the liver cells. Explain this observation.

THE DUPLICATION OF THE GENOME MILLIONS OF YEARS AGO MEANS THAT THERE WILL BE MUTATIONS IN THE DNA. SOME IN THE 3RD NUCLEOTIDE OF CODONS WILL NOT CHANGE THE AMINO ACID SEQUENCE, OTHERS IN NON-ESSENTIAL AMINO ACIDS WILL NOT BE SELECTED AGAINST. RNAi IS VERY SELECTIVE FOR THE mRNA COMPLEMENTARY TO THE RNAi OLIGONUCLEOTIDE. SINCE ONLY ONE CLONE WAS USED, THE SEQUENCE MAY DIFFER IN THE ALBUMIN mRNAs MADE FROM THE DUPLICATED DNA. IF THE SEQUENCE DIFFERS THE RNAi WILL NOT ANNEAL CORRECTLY AND INDUCE DEGRADATION. SINCE HALF AS MUCH PROTEIN WAS MADE, IT IS LIKELY THAT ONE SET OF ALBUMIN mRNAs WAS COMPLEMENTARY TO THE RNAi AND WAS RNAi AND WAS DEGRADED AND OTHER HALF OF THE mRNAs AND WERE NOT DUPLICATED (we discussed this type of issue when we talked about the sequence conservation of nuclear receptor DNA binding domains)

5. Histones repress transcription. Modification of lysine side chains on the amino acids in the N-terminal region of histone H3 by acetylation or methylation is associated with relief of histone repression of transcription. To get rid of these positively charged amino acids side chains entirely, a student deleted the entire N-terminal 30 amino acids from histone H3, and produced large amounts of mutant histone H3. The mutant histone H3, or the wild-type histone H3, and the other wild-type histones were used to reconstitute correctly spaced nucleosomes on a test promoter containing thyroid hormone response elements (TREs) in the 5'-region upstream of the transcription start site. Transcription was then studied in a system containing a crude HeLa cell nuclear extract that supports regulated cell-free transcription. This extract lacks thyroid receptor. Thyroid hormone and thyroid receptor were added to initiate high level transcription of the test promoter. As expected, forming nucleosomes containing the mutant histone H3 or wild-type histone H3 repressed basal transcription of the test promoter. When thyroid hormone and thyroid receptor were added to the templates reconstituted with the wild-type Histone H3, there was a large increase in transcription. In contrast, when thyroid hormone and thyroid receptor were added to the templates reconstituted with the mutant histone H3 lacking the N-terminal 30 amino acids there was very little increase in transcription.

IN THE HISTONE CODE MODEL FOR GENE TRANSCRIPTION HISTONE SIDE CHAIN MODIFICATIONS ARE RECOGNIZED BY CELL PROTEINS THAT ENHANCE ACTIVATION OR REPRESSION. ACETYLATION AND METHYLATION ARE RECOGNIZED BY PROTEINS THAT FURTHER MODIFY HISTONES AND BY HISTONE REMODELING COMPLEXES THAT REORGANIZE NUCLEOSOMES. BY REMOVING THE HISTONE TAILS, THE INVESTIGATOR REMOVED MOST OF THE SEQUENCES THAT PROVIDE SITES FOR MODIFICATIONS RECOGNIZED BY PROTEINS IMPORTANT IN ACTIVATING TRANSCRIPTION. THEREFORE THE PRESENCE OF THE MUTANT HISTONE CAUSES POOR RECRUITMENT OF OTHER PROTEINS AND MACROMOLECULAR COMPLEXES TO THE CHROMATIN AND THERE IS POOR RELIEF OF CHROMATIN REPRESSION. (These sequence modifications were mentioned in the table in the handout)