

Bio 366: Biological Chemistry II
Test #3, 100 points

READ THIS: Take a numbered test and sit in the seat with that number on it. Remove the numbered sticker from the desk, and stick it on the back of the last page of the test. Print the last four digits of your social security number on the back of the test, and your name on the front top of each page. When you have finished, hand in your test and sign your name on the sign-out sheet by the door. The answers will be posted outside of my office (Bio 229) within a day or two. If you wish to challenge an answer, give me a written explanation as soon as possible. If you wish to question the grading of this exam, you must also give me a written explanation, and hand it back to me with your whole test, which will be completely regraded (*i.e.*, you could lose points).

1. [8 points total; 2 points for each step] Describe the pathway for the base excision repair of damaged DNA. Include each step of the pathway, starting from the recognition of the damaged base to its final restoration. Be sure to specify which enzyme is involved in each step, and describe the substrate and product of each reaction. (Examples of each enzyme type are unnecessary.)

2. Match the enzyme/protein/process [1.5 points each; 30 points total]: Write the correct number(s) in the blank next to the statement. Some have more than one correct answer; list them all for full credit. The same letter may be used for more than one answer, or may not be used at all.

KEY:

1. Ada protein
2. Aminoacyl-tRNA synthetase, Class I
3. Aminoacyl-tRNA synthetase, Class II
4. Dam methylase
5. Deoxyribosephosphodiesterase
6. DNA ligase
7. DNA helicase II (Uvr D)
8. *E. coli* DNA polymerase I
9. *E. coli* DNA polymerase II
10. *E. coli* DNA polymerase III
11. *E. coli* RNA polymerase "core enzyme"
12. *E. coli* RNA polymerase "holoenzyme"
13. Endonuclease III
14. Endonuclease IV
15. Exonuclease III
16. Formamidopyrimidine-DNA glycosylase
17. Guanylyl transferase
18. HIV reverse transcriptase
19. Homeobox domain protein
20. Inorganic pyrophosphatase
21. 3-Methyl adenine DNA glycosylase
22. Mut Y
23. Photolyase
24. Poly (A) polymerase
25. Primase
26. Pyrimidine dimer DNA glycosylase
27. RNase H
28. RNA polymerase I
29. RNA polymerase II
30. RNA polymerase III
31. Rho factor
32. Sigma factor
33. Thymine mismatch DNA glycosylase
34. Topoisomerase
35. Transcription factor
36. Uracil-DNA glycosylase
37. none of the above

- A. _____ A DNA repair enzyme that transfers a methyl group from O⁶-methyl guanine or O⁴-methyl thymine to one of its own cysteine residues.
- B. _____ Helps render many biochemical reactions essentially irreversible.
- C. _____ Catalyzes the removal of uracil from DNA.
- D. _____ Is localized to the nucleolus, and transcribes the major rRNA genes
- E. _____ Most are monomeric proteins, and all add amino acids to the 2'-OH of the 3'-terminal adenine of their cognate tRNAs.
- F. _____ A DNA-binding protein that recognizes a specific promoter sequence and accurately initiates transcription in eukaryotes.
- G. _____ Enzymes that initiate the repair of abasic sites in DNA.
- H. _____ Completes the nucleotide excision repair process in prokaryotes by sealing nicks in DNA.
- I. _____ A DNA-binding protein that contains a helix-turn-helix motif.
- J. _____ All cells have 10 members of this class of proteins, which are needed for protein synthesis.
- K. _____ Eukaryotic cells uses this enzyme(s) to synthesize RNAs.
- L. _____ Recognizes promoter sites in prokaryotic genes.
- M. _____ Has a turnover number of about 9,000 nucleotides polymerized per minute at 37°C, and appears to be the *E. coli* DNA replication enzyme.
- N. _____ The drug, AZT (3'-azido-2',3'-dideoxythymidine), binds preferentially to this enzyme.
- O. _____ Removes the thymine in T-G mismatches of DNA.
- P. _____ Has RNA-directed DNA polymerase, DNA-directed DNA polymerase, and RNase H (ribonuclease) activities.
- Q. _____ Couple together ribonucleoside triphosphates (ATP, CTP, GTP and UTP) on DNA templates.
- R. _____ Dissociates from the core enzyme once RNA synthesis is initiated.
- S. _____ Has 5'→3' polymerase, 5'→3' exonuclease, and 3'→5' exonuclease activities.
- T. _____ These proteins always recognize their cognate tRNAs by binding contacts with the anticodons.

3. True or False. Circle the correct answer. (1 point each, 25 points total.)

- a. T F Ribosomes are found only in the cytoplasm of cells.
- b. T F During translation, the codons in mRNA are recognized by aminoacyl-tRNAs.
- c. T F All DNA polymerases can initiate DNA synthesis *de novo*.
- d. T F Meselson and Stahl showed that DNA replication is conservative.
- e. T F All tRNA molecules, whether of eukaryotic or prokaryotic origin, have the sequence CCA at their 3' ends.
- f. T F The "nick translation" activity of *E. coli* DNA polymerase I results in *net* synthesis of new DNA.
- g. T F The Cx classes of zinc-finger proteins coordinate the zinc ion using histidine and cysteine side chains.
- h. T F *E. coli* DNA polymerase I has its own "proofreading" and "editing" functions.
- i. T F You have printed your name at the top of each page and the last four digits of your social security number on the back of the last page. (This should be TRUE, but if you do not do this, you will lose the point!)
- j. T F Only 2'-OH aminoacyl-tRNAs are substrates for protein synthesis.
- k. T F Deamination of cytosine in DNA is premutagenic.
- l. T F Like most other biological polymerization processes, protein synthesis is characterized by three phases: initiation, elongation, and termination.
- m. T F The energy driving protein synthesis is provided by ATP.
- n. T F Prokaryotic RNA polymerase binds nonspecifically to DNA with low affinity, then migrates along it searching for a promoter.
- o. T F Both base excision repair and nucleotide excision repair can play a role in the repair of thymine dimers in DNA.
- p. T F Termination of protein synthesis is an imprecise process.
- q. T F Most *E. coli* promoters have consensus sequences at the transcription initiation site (+1 in the sequence), the "Pribnow box" (at about -10), and a region at about -35 from the transcription start site.
- r. T F In prokaryotes, virtually all RNA types are synthesized by a single species of DNA-dependent (a.k.a., DNA-directed) RNA polymerase.
- s. T F Most eukaryotic cells have several different DNA polymerases in the nucleus, and a separate DNA polymerase in the mitochondria.
- t. T F Reactive oxygen species can be generated by ionizing radiation, by normal aerobic respiration, and by various drugs.
- u. T F Xeroderma pigmentosum is a genetic disease associated with a deficiency in nucleotide excision repair.
- v. T F DNA polymerase I of *E. coli* plays a major role in base excision repair, nucleotide excision repair, and strand-specific mismatch repair.
- w. T F The degree to which an enzyme remains associated with template during successive rounds of polymerization is referred to as its "processivity".
- x. T F The Klenow fragment of *E. coli* DNA polymerase I contains the so-called "nick-translation" activity, which provides a useful tool for radioactively-labeling DNA in the laboratory.
- y. T F No primer is needed during RNA tumor virus replication.

4. [5 points] Draw a simplified "cloverleaf" structure of an aminoacylated tRNA^{ala} molecule, showing its pairing to a codon for alanine. [For full credit, you do not need to show the structures of the individual nucleotides involved in the base pairs, but do need to show the correct (5' or 3') orientation of the anticodon and codon.] Explain the structural basis of the "wobble".

5. [5 points] Fill in the following table:

Allowed Wobble Pairing Combinations in the Third Codon-Anticodon Position	
5'-anticodon base	3'-codon base
	U or C
	U
	A or G
	U, C or A
	G

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6. [18 points total, 6 points for each part] Describe the post-transcriptional processing of mRNA precursors in eukaryotic cells. Include explanations of i) CAPPING and METHYLATION (with structures for full credit), ii) POLYADENYLATION, and iii) SPLICING (include discussion of the consensus sequences and diagram the mechanism). You may use two pages to answer this 3-part question, but be succinct!

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Continuation page for question 6.

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7. [9 points] Explain how the Sanger (or dideoxy) DNA sequencing method works *at the biochemical level*. Your answer should include a description of the DNA polymerization process, illustrating the enzymatic reaction.