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EXAM IV
December 7, 1998
Biochemistry I
BI/CH421, BI601, BI/CH621

I. _____/ 60

II. _____/ 15

III. _____/ 13

IV. _____/ 12

V. _____/ 10(grads)

TOTAL /100 or 110

I. **MULTIPLE CHOICE**. (60 points; first 14 are 3 pts the last 9 are 2 pts) Choose the BEST answer to the question by circling the appropriate letter.

1. Certain restriction enzymes produce cohesive (sticky) ends. This means that they:

   A. cut in regions of high GC content, leaving ends that can form more hydrogen bonds than ends of high AT content.
   B. make a staggered double-strand cut, leaving ends with a few nucleotides of single-stranded DNA protruding.
   C. cut both DNA strands at the same base pair.
   D. stick tightly to the ends of the DNA it has cut.
   E. have none of the above characteristics.

2. Which of the following statements correctly describes promoters in E. coli?

   A. All promoters have the same sequence, which is that recognized by RNA polymerase holoenzyme.
   B. Every promoter has a different sequence, with little or no resemblance to other promoters.
   C. Many promoters are similar and resemble a consensus sequence, which has the highest affinity for RNA polymerase holoenzyme.
   D. A promoter may be present on either side of a gene or in the middle of it.
   E. Promoters are not essential for gene transcription, but they can increase transcription by two- to threefold.

3. It is possible to convert the Cys that is a part of Cys-$tRNA^{Cys}$ to Ala by a catalytic reduction. If the resulting Ala-$tRNA^{Cys}$ were added to a mixture of ribosomes, all the other tRNAs and amino acids, all of the cofactors and enzymes needed to make protein in vitro, and mRNA for hemoglobin, where in the newly synthesized hemoglobin would the Ala from Ala-$tRNA^{Cys}$ be incorporated?

   A. wherever Ala normally occurs
   B. wherever Cys normally occurs
   C. wherever either Ala or Cys normally occurs
   D. wherever the dipeptide Ala-Cys normally occurs
   E. nowhere; this is the equivalent of a nonsense mutation

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4. The Watson-Crick base pairing scheme for an A-T base pair includes:

   A. a hydrogen bond between a keto oxygen and an extracyclic amino group.
   B. a hydrogen bond between two ring nitrogen atoms.
   C. an ionic bond between the positively charged adenine amino group and a negatively polarized keto group.
   D. both A and B.
   E. both B and C.

5. In a Watson-Crick base pair for an A-T, how would the hydrogen bonds change if the adenine base were in its imine tautomer?

   A. The extracyclic imino group would become a hydrogen bond acceptor.
   B. The three hydrogen bonds to thymidine would break.
   C. The methyl group of adenine would make hydrophobic contact with thymine
   D. The two hydrogen bonds to thymidine would break.
   E. The keto oxygen would become a hydrogen bond donor as a hydroxyl.
   F. both A and D
   G. both A and E

6. In the chemical synthesis of DNA:

   A. the nucleotide initially attached to the silica gel support will become the 3' end of the finished product.
   B. the dimethoxytrityl (DMT) group catalyzes formation of the phosphodiester bond.
   C. the maximum length of oligonucleotide that can be synthesized is 8-10 nucleotides.
   D. all of the above are correct.
   E. none of the above are correct.

7. When double-stranded DNA is heated at neutral pH, which change does **not** occur?

   A. The absorption of ultraviolet (260nm) light increases.
   B. The covalent N-glycosidic bond between the base and the pentose breaks.
   C. The hydrogen bonds between A and T break.
   D. The viscosity of the solution decreases.
   E. The helical structure unwinds.

8. Which of the following does **not** apply to the construction or use of a DNA library?

   A. Many segments of DNA from a cellular genome are cloned.
   B. Specialized DNA libraries can be made by cloning DNA copies of mRNAs.
   C. The DNA copies of mRNA found in a cDNA library are made by reverse transcriptase.
   D. Each plasmid must contain Pol genes.
   E. Finding a particular DNA sequence in a DNA library can be done using a suitable hybridization probe.

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9. Which of the following statements is true?

   A. Exonucleases degrade DNA at a free end.
   B. Endonucleases degrade circular but not linear DNA molecules.
   C. Many DNA polymerases have a proofreading 5' → 3' exonuclease.
   D. *E. coli* DNA polymerase I is unusual in that it possesses only a 5' → 3' exonucleolytic activity.

10. The artificial mRNA polyadenylic acid (poly(A)) can be prepared in the laboratory by incubating a mixture containing:

   A. DNA, ATP, and an RNA polymerase.
   B. UTP and an RNA polymerase.
   C. RNA, Pi, and polynucleotide phosphorylase.
   D. ATP and polynucleotide phosphorylase.
   E. ADP and polynucleotide phosphorylase.

11. DNA supercoiling:

   A. results in compaction of the DNA structure.
   B. is induced by the process of DNA replication.
   C. is induced by the process of RNA synthesis (transcription).
   D. has all of the above characteristics.

12. Formation of the 70S ribosomal initiation complex for bacterial protein synthesis does *not* require:

   A. formylmethionyl $tRNA^{fMet}$.
   B. initiation factor 2 (IF-2).
   C. GTP.
   D. mRNA.
   E. EF-Tu.

13. The enzyme that attaches an amino acid to a tRNA (aminoacyl-tRNA synthetase):

   A. always recognizes a specific tRNA.
   B. catalyzes formation of an ester bond.
   C. attaches a specific amino acid to any available tRNA species.
   D. attaches the amino acid at the 5' end of the tRNA.
   E. splits ATP to ADP + P_i.

14. Which of the following statements about tRNA molecules is **false**?

   A. There is at least one tRNA for each of the 20 amino acids.
   B. The amino acid attachment is always to an A nucleotide at the 3' end of the molecule.
   C. Any given tRNA will normally only accept one specific amino acid.
   D. A, C, G, and U are the only bases present in the molecule.
   E. Although composed of a single strand of RNA, each molecule contains several short, double-helical regions.

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15. In living cells nucleotides serve as:

A. precursors for nucleic acid synthesis.
B. enzyme cofactors.
C. intracellular signals.
D. carriers of metabolic energy.
E. all of the above.

16. RNA polymerase:

A. separates DNA strands throughout a long region of DNA (up to thousands of base pairs), then copies one of them.
B. has a subunit called λ (lambda), which acts as a proofreading ribonuclease.
C. binds tightly to a region of DNA thousands of base pairs away from the DNA to be transcribed.
D. can synthesize RNA chains de novo (without a primer).

17. Topoisomerases can:

A. change the number of base pairs in a DNA molecule.
B. change the number of nucleotides in a DNA molecule.
C. change the linking number (Lk) of a DNA molecule.
D. convert D isomers of nucleotides to L isomers.
E. do none of the above.

18. The fundamental repeating unit of organization in a eukaryotic chromosome is:

A. the centrosome.
B. the nucleosome.
C. the lysosome.
D. the microsome.
E. none of the above.

19. Which of the following deoxyoligonucleotides will hybridize with a DNA containing the sequence (5')AGACTGGTC(3')?

A. (5')TCTGACCAG(3')
B. (5')GAGTCAACT(3')
C. (5')CTCATTGAG(3')
D. (5')GACCAGTCT(3')
E. (5')TCTGGATCT(3')

20. The compound that consists of ribose linked by an N-glycosidic bond to N-9 of adenine is:

A. a purine nucleotide.
B. a pyrimidine nucleotide.
C. adenosine.
D. AMP.
E. a deoxyribonucleoside.

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21. The nucleic acid bases:

A. absorb ultraviolet (260 nm) light.
B. are roughly planar.
C. are relatively hydrophobic.
D. have all of the above characteristics.
E. have none of the above characteristics (A-C).

22. The elongation stage of protein synthesis does **not** involve:

A. GTP.
B. peptidyl transferase.
C. EF-Tu.
D. IF2.
E. aminoacyl-tRNAs.

23. The Meselson-Stahl experiment established:

A. that newly synthesized DNA in E. coli has a different base composition than the preexisting DNA.
B. the role of DNA polymerase in DNA synthesis.
C. that DNA synthesis in E. coli proceeds by a conservative mechanism.
D. that DNA synthesis requires dATP, dCTP, dGTP, and dTTP.
E. that the two strands of parental DNA separate during replication in E. coli.

II. **SHORT ANSWER** (15 points)
Give a brief answer to each problem or question below.

24. Explain how each of the following is used in cloning a plasmid: (a) antibiotic resistance genes; (b) origin of replication; (c) polylinker region (multiple unique restriction sites). (3 pts)

25. Nucelotide polymerization is a slightly endergonic reaction ($\Delta G^{\circ\prime} \approx 8$ kcal/mol), yet this reaction proceeds spontaneously in the cell. Explain. (2 pts)

26. In a ρ-independent terminator, there is a palindrome rich in G≡C base pairs, followed by 8-10 uridine residues. Explain how each of the following changes might affect terminator function:
(a) Substitution of cytidines for the 8-10 uridines.
(b) Mutations in the palindrome that decreased its G≡C content.
(c) Elimination of half of the palindromic sequence. (3 pts)

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27. In one sentence, identify the most obvious structural difference between B-form (Watson-Crick) DNA and Z-form DNA. (2 pts)

28. A suitable substrate for DNA polymerase is shown below. Label the primer and template, and indicate which end of each strand must be 3' or 5'.

To observe DNA synthesis on this substrate in vitro, what additional reaction components must be added? (5 pts)

III. **MATCHING & FILL-IN-THE-BLANK** (13 points)

29. Match the type of bond with the role below:

Bond type	Role
(a) phosphodiester	___ links base to pentose in nucleotide
(b) N-glycosidic	___ joins adjacent nucleotides in one strand
(c) phosphate ester	___ joins complementary nucleotides in two strands
(d) hydrogen	___ difference between a nucleoside and a nucleotide.

30. DNA synthesis on the lagging strand in E. coli is a complex process known to involve several proteins. Initiation of a new chain is catalyzed by the enzyme (a) _____, and elongation is catalyzed by the enzyme (b)_____. Synthesis is discontinuous, yielding short segments called (c) _____, which are eventually joined by the enzyme (d)_____, which requires the cofactor (e)_____.

31. Place the following steps (1-4) in the proper order with regard to protein synthesis.

___ Peptide bond formation shifts the growing peptide from the P to the A site.
___ Charged tRNA binds to the A site.
___ The 50S subunit binds to the initiation complex of the 30S subunit and mRNA.
___ Uncharged tRNA is released from ribosome.

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IV. **PROBLEMS** (12 points)

32. After her adventures in Oz, Dorothy came to BU and became a biochemist. Soon thereafter, she was summoned back to Oz by the wizard to carry out a special project. The wizard, as it happens, had been dabbling in biochemistry himself. He had determined that all the basic rules of protein and nucleic acid structure and synthesis are the same in Oz as they are on earth, with only two apparent exceptions. First, in Oz, only 12 different amino acids could be detected in protein samples (Gly, Pro, Leu, Lys, Arg, Phe, Tyr, Glu, Ser, Cys, Gln, and Met). Second, the wizard discovered that the genetic code in Oz was a doublet rather than a triplet code. He wanted Dorothy to solve the genetic code for Oz and she agreed to try. Employing a local bacteria, Yellowus brickus, she set up a cell-free protein synthesis system. She then synthesized a number of RNA polymers using both chemical and enzymatic methods. She added the synthetic RNA molecules to her cell-free protein synthesis system and analyzed the amino acid content of the resulting polypeptides. These experiments yielded a complete answer in a few months time. Already possessing a perfectly adequate brain, Dorothy demanded hard cash for her efforts. The wizard not only complied, but kept her on the payroll to work on suicide inhibitors for wicked witches. Our story ends here, but yours does not. Dorothy's results are given below. Use these results to work out the genetic code for Oz and fill in the table provided. **IMPORTANT**: Assume that any degeneracy in the code occurs only in the second base of the codon (i.e., if two codons code for the same amino acid, the first base of both codons is identical).

Dorothy's Results

Synthetic RNA	Amino acid composition of polypeptide product
poly(A)	Gly 100%
poly(U)	Lys 100%
poly(C)	Tyr 100%
poly(A) with one U at 3' end	Gly 100%
poly(U) with one A at 3' end	Lys 100%
poly(C) with one G at 3' end	Tyr 96%, Pro 4%
AUAUAUAU etc.	Gly 100%
ACACACAC etc.	2 different polypeptides: Phe 100%, or Met 100%
80% U, 20% G	Lys 63%, Leu 16%, Cys 17%, Gln 4%
80% A, 20% G	Gly 65%, Arg 16%, Leu 15%, Gln 4%
80% C, 20% U	Tyr 64%, Ser 16%, Phe 17%, Lys 3%
80% C, 20% G	Tyr 62%, Glu 17%, Pro 18%, Gln 3%

A.

The Genetic Code of Oz

Second Base in codon

First base in codon	A	C	U	G
A				
C				
U				
G				

B. Knowing that the stop codon(s) is determined only by indirect inference, what sequence could you synthesize in a more defined polymer and use in this experimental system that would support your identification of the stop codon(s) on Oz? Explain.

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V. **Graduate Students ONLY** (10 pts)

33. You have isolated a fragment of viral DNA that totally encodes at least two proteins, 120 and 80 amino acids long. The DNA fragment is 400 base pairs long. (a) Why might you consider this unusual? (b) Propose two models to account for this, one of which you might be able to rule out if you sequence the two proteins. (c) You sequence the two proteins and find no sequence homology. Which proposal does this rule out? Why? (7 pts)

34. Briefly describe one of the experimental methods that gave evidence that the genetic code is a triplet code. (3 pts)

Answer Sheet for Exam 4, 12/7/98

Question	Correct Answer
Multiple Choice	
1	B
2	C
3	B
4	D
5	F
6	A
7	B
8	D
9	A
10	E
11	D
12	E
13	B
14	D
15	E
16	D
17	C
18	B
19	D
20	C
21	D
22	D
23	E
Short Answer	
24	(a) Antibiotic resistance allows a researcher to select for a bacterial clone carries the plasmid; loss of an antibiotic marker in a strain known to contain the plasmid can be used to infer the presence of a cloned DNA segment that interrupts the antibiotic resistance gene. (b) An origin of replication assures that the plasmid will replicate autonomously in the bacterium. (c) Polylinker has cut sites for a variety of restriction enzymes, allowing insertion of DNA fragments produced with any of them.
25	In the cell, pyrophosphatase couples polymerization to the highly exergonic hydrolysis of the pyrophosphate product such that the back reaction has little pyrophosphate available for reversal.
26	(a) This substitution would decrease terminator function by stabilizing the RNA-DNA hybrid duplex. (b) These mutations would decrease terminator function by destabilizing hairpin formation, and the RNA-DNA hybrid will be stabilized as a result. (c) Without half the palindrome, the hairpin will not form, and the RNA-DNA hybrid will not be destabilized enough for the terminator to function.
27	A-form has a right-handed helix; Z-form has a left-handed helix.
28	The top strand (the primer) has its 5' end to the left; the bottom (template) strand has the opposite polarity.

Question	Correct Answer
28 (continued)	For in vitro DNA synthesis with this substrate, one would have to add DNA polymerase and the four deoxynucleoside triphosphates.
Matching/FillIn	
29	b; a; d; c
30	(a) primase; (b) DNA pol III; (c) Okazaki fragments; (d) DNA ligase; (e) NAD^+(or ATP)
31	3; 2; 1; 4
Problems	
32	A. The Genetic Code of Oz

Second Base in codon

First base in codon	A	C	U	G
A	Gly	Met	Gly	Arg
C	Phe	Tyr	Phe	Pro
U	stop	Ser	Lys	Cys
G	Leu	Glu	Leu	Gln

B. Use a polymer such as (UAAC)n that contains the stop codon.

GRADS ONLY 33. (a) Two distinct proteins of these sizes should require mRNAs of 360 and 240 base pairs, because each amino acid residue requires 3 base pairs to code for it. (b) One possible explanation is that the two genes coding for these proteins overlap and are read in different reading frames. Another is that the gene encodes a protein of 120 residues that is proteolytically cleaved to yield the protein of 80 amino acid residues. (c) No homology means that the smaller protein cannot be derived from the larger by proteolysis; if it were, there would be 80 amino acid residues of identical sequence in the two proteins.

34 When one or two nucleotides were added to or deleted from a gene, the resulting frameshift scrambled the sequence after the deletion or insertion. When three nucleotides were added or deleted, the resulting protein had a normal sequence except for the insertion or deletion or a single amino acid residue; the reading frame was not shifted.