	NAME	· · · · · · · · · · · · · · · · · · ·
Exam IV	I.	/28
Biochemistry I BI/CH 421/621	II.	/10
	III.	/49
	IV.	/13
	TOTAL	/100

I. <u>MULTIPLE CHOICE</u>. (28 points; 2 points each) Choose the BEST answer to the question by circling the appropriate letter.

- 1. The phosphodiester bonds that link adjacent nucleotides in both RNA and DNA:
  - A. join the 3' hydroxyl of one nucleotide to the 5' hydroxyl of the next.
  - B. are susceptible to alkaline hydrolysis.
  - C. are uncharged at neutral pH.
  - D. always link A with T and G with C.
- 2. 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
- 3. At pH 7.0, the rare protonation of adenine at N1  $(pK_a=3.8)$  can lead to:
  - A. deprotonation of N7
  - B. base pairing with thymidine
  - C. A:T  $\rightarrow$  G:C mutations
  - D. A:T  $\rightarrow$  A:G mutations
  - E. A:T  $\rightarrow$  T:A mutations
- 4. Which of the following statements about DNA ligase is false?
  - A. It forms a phosphodiester bond between a 5'-hydroxyl and a 3'phosphate in duplex DNA.
  - B. It requires a cofactor, either NAD<sup>+</sup> or ATP, depending on the source of the enzyme, to provide the energy to form the phosphodiester bond.
  - C. It catalyzes its reaction by a mechanism that involves the formation of a covalently linked enzyme adenylate.
  - D. It catalytzes its reaction by a mechanism that involves the activation of a DNA phosphate through the formation of a phosphoanhydride bond with AMP.
  - E. It is involved in DNA replication and used in rDNA experiments.

- 5. The production of pyrophosphate, which is immediately hydrolyzed to two molecules of inorganic phosphate by pyrophosphatase, is the key reason that is/are not readily reversible in the cell.
  - A. the production of DNA by DNA polymerase
  - B. the sealing of a nick by DNA ligases that use ATP
  - C. the production of the peptide bonds by the ribosome
  - D. the production of RNA by RNA polymerase
  - E. all of the above
  - F. A, B, & C
  - G. A, B, & D
  - H. A & D
- 6. A convenient cloning vector with which to introduce foreign DNA into E. coli is a(n):
  - A. yeast transposable element.
  - B. E. coli chromosome.
  - C. plasmid.
  - D. yeast "ARS" sequence.
  - E. messenger RNA.
- 7. Processing of a primary mRNA transcript in a eukaryotic cell does *not* normally involve:
  - A. attachment of a long poly(A) sequence at the 3'-end.
  - B. methylation of one or more guanine nucleotides at the 5'-end.
  - C. excision of intervening sequences (introns).
  - D. conversion of normal bases to modified bases such as inosine and pseudouridine.
  - E. joining of adjacent exons.
- 8. Compared with DNA polymerase, reverse transcriptase:
  - A. makes fewer errors in synthesizing a complementary polynucleotide.
  - B. makes more errors, because it lacks the 3'  $\rightarrow$  5' proofreading exonuclease activity.
  - C. introduces no errors into genetic material, because it synthesizes RNA, not DNA.
  - D. synthesizes complementary strands in the opposite direction--from  $3\,{}^\prime\!\rightarrow\!5\,{}^\prime$  .
  - E. has none of the above characteristics.
- 9. All of the following contribute to promoter binding by RNA polymerase in *E. coli* EXCEPT the
  - A. rho factor
  - B. -10 consensus sequence
  - C. -35 consensus sequence
  - D.  $\sigma$  subunit of RNA polymerase
  - E.  $\beta$  subunit of RNA polymerase

- 10. Which of the following statements about the elongation phase of protein synthesis is *false*?
  - A. Peptidyl transferase is a ribozyme.
  - B. Incoming aminoacylated tRNAs are first bound in the A site.
  - C. Peptidyl transferase catalyzes the attack of the  $\alpha$ -amino group of the amino acid in the A site on an ester linkage in the P site.
  - D. Elongation factor EF-Tu facilitates translocation.
  - E. At least four high-energy phosphoryl groups are expended for each peptide bond formed (including only tRNA activation and translation).
- 11. Which of the following statements describes fatty acids?
  - A. Phosphatidic acid is a common one.
  - B. They are strongly hydrophilic.
  - C. Some are precursors of prostaglandins.
  - D. They are a constituent of sterols.
- 12. Which of the following statements about sterols is true?
  - A. Sterols are found in the membranes of all living cells.
  - B. The principal sterol of animal cells is ergosterol.
  - C. All sterols share a fused-ring structure with four rings.
  - D. Sterols are very soluble in water, but less so in organic solvents such as chloroform.
- 13. All of following statements about the fluid mosaic model of biological membranes are true EXCEPT:
  - A. Lipid molecules in the membrane readily undergo lateral diffusion.
  - B. Lipid molecules in the membrane readily undergo transverse (flip-flop) diffusion.
  - C. Integral membrane proteins can undergo lateral diffusion.
  - D. The saturated hydrocarbon chains of lipid molecules in the membrane undergo carbon-carbon bond rotation.
  - E. The transition temperature of a membrane is sensitive to the composition of the lipid molecule in the membrane.
- 14. The enzyme ATCase showed a molecular weight of ca. 300 kDa in a gel filtration experiment. In another experiment ATCase was run on SDS PAGE and two bands were seen, one ( $\alpha$ ) at 34 kDa and one ( $\beta$ ) at 17 kDa. The 34 kDa band stained darker than the 17 kDa band. What is the quaternary structure of ATCase?
  - A.  $\alpha_2\beta_1$
  - **Β.** α<sub>6</sub>β<sub>6</sub>
  - c.  $\alpha_4\beta_4$
  - D.  $\alpha_8\beta_4$

II. **STRUCTURES**. (10 points)

15. Draw the structure of 1-palmitoyl, 2-oleoyl, phosphatidylserine in the ionic form it would have at pH 7. (6 pts)

16. Draw the structure of Cholesterol. Circle the isoprene units involving carbons 16, 17, 20-27. (4 pts)

III. <u>SHORT ANSWER</u>. (49 points)
Give a <u>brief</u> answer or diagram for each problem or question below.

- 17. Write out the Central Dogma of Molecular Biology. Include the name of the processes for each information-transfer step and the the principle enzyme and or ribozyme involved in each step. (3 pts)
- 18. Write the sequence of the messenger RNA molecule synthesized from a DNA template strand having the sequence: (2 pts) (5')ATCGTACCGTTA(3')
- 19. Name three levels of gene regulation that might be use to increase or decrease the concentration of an enzyme. (3 pts)
- Name one enzyme that is always used to make a cDNA library, but is not generally used to make a genomic DNA library. Describe its function briefly. (3 pts)

21. Compare and contrast molecular cloning and polymerase chain reaction (PCR). (3 pts)

- 22. RNA is readily hydrolyzed by alkali, whereas DNA is not. Why? (2 pts)
- 23. What is the difference between the <u>data</u> in a file or record from the GenBank database versus that from the Protein database? (2 pts)
- 24. Explain why DNA rich in G/C base pairs has a higher  $T_m$  than DNA that is rich in A/T base paris. (5 pts)
- 25. Describe which <u>enzymatic activities</u> (e.g., dehydrogenase, reductase, kinase, etc.) are responsible for the fidelity of each of the following and what the degree of fidelity is (e.g., 1 mistake in 100 events = 10<sup>-2</sup>). (8 points)
  - 1. DNA replication
  - 2. Transcription
  - 3. tRNA activation
  - 4. Translation
- 26. A hypothetical enzyme AMAse can be purified from brain (B) and liver (L). Upon cellulose acetate electrophoresis at pH 5.5, the preparation from brain has a mobility of +5 and that from liver a mobility of +35. Each preparation gives a single band. If the two preparations are mixed and incubated at 37 °C for half an hour and then run on electrophoresis, the resulting zymogram shows a total of seven bands, including two which have the same mobility as the original two enzymes. The other five bands are spaced evenly between the original bands. Explain these results in terms of the quaternary structure of AMAse. (3 pts)

- 27. 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 decreases effectiveness of terminator function:
  (a) Substitution of cytidines for the 8-10 uridines. (3 pts)
  - (b) Mutations in the palindrome that decreased its G=C content.
  - (c) Elimination of half of the palindromic sequence.
- 28. What are the chemical components of a biological wax, and what is their general structure? (2 pts)
- 29. (a) Discuss the differences in structure and chemical properties between triacylglycerols and glycerolphospholipids. (b) Which type of lipid discussed above is capable of spontaneously assembling into the bilayer structure found in biological membranes? (c) What are the forces that drive bilayer formation? (4 pts)

30. Name two experiments or observations that disproved the old membrane structure model and two experiments that support the <u>fluid</u> mosaic model for membrane structure. (4 pts)

31. A new antibiotic named ericamycin was recently discovered by a brilliant graduate student. This antibiotic works by inhibiting prokaryotic protein synthesis. In the presence of ericamycin protein synthesis can initiate, but only dipeptides that remain bound to the ribosome are formed. What specific step of protein synthesis is likely to be blocked by this antibiotic? (2 pts)

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## MATCHING. (13 points) TV.

- Match the critical discoveries or theories on the left with the main proponent/ 32. scientist who was responsible on the right. (6 pts)
- 1. Discovered traits and their inheritance
- 2. Elucidated the structure of DNA
- Collected critical data for determination of the 3. structure of DNA
- 4. Discovered DNA

12.

- 5. Showed that DNA carried heredity
- 6. Base composition of DNA
- 7. Proposed the existance of mRNA
- 8. Proposed the existance of tRNA
- 9. Proposed the semi-conservative replication of DNA
- 10. Proved semi-conservative replication of DNA
- Proposed the lagging strand replication 11. Showed that the genetic code is triplet
- Meselson & Stahl D.

Α.

в.

C.

Ε. Mendel

Crick

Okazaki

- F. Chargaff
- G. Miescher
- H. Avery & McCarty

*Ram*H I

Sal I

(insert)

I. Rosalind Franklin

Watson & Crick

33. Match each feature of the bacterial expression plasmid (at left) with one appropriate description presented (at right) (see picture below). (3 pts)

(smp<sup>R</sup>

ari)

promoter

- (a) permits selection of bacteria containing
  - the plasmid
- ori sequence (b) a sequence containing an open-reading frame insert sequence

Pvu II ~

- BamH I & Sal I sites (c) origin of replication

amp<sup>R</sup> sequence

- promoter sequence
- Pst I & Pvu II sites (d) cleavage of the plasmid here might affect the antibiotic resistance genes
  - (e) permits the expression of a protein
  - (f) cleavage of the plasmid here was used to make this recombinant plasmid
- 34. Match the factor or enzyme at right with the stage(s) of protein synthesis at which it acts. If a factor or enzyme participates in two stages of protein synthesis, indicate all of them. (4 pts)
  - amino acid activation
  - initiation
  - elongation
  - translation termination
- (a) RF1 (b) EF-Tu

- (c) AA-tRNA synthetase (d) Shine-Dalgarno sequence

No. on	L	
Test	Correct	Answer
1	A	
2	F	
3	С	
4	A	
5	G	
6	C	
7	D	
8	В	
9	A	
10	D	
11	C	
12	C P	
13	B	
14	В	
15		1-palmitoyl, 2-oleoyl phosphatidylserine
	16:0	
	18:1	O C-O-CH <sub>2</sub> C-O-CH <sub>2</sub> C-O-CH <sub>2</sub> CH <sub>2</sub> O-P-O-CH <sub>2</sub> -CH-COO- O-

At neutral pH there is a charge on the phosphate group, and serine is in the zwitterionic form; it has a protonated amino group and an ionized carboxyl group.

16



17

DNA -----> RNA ----> Protein

with DNA getting more of itself in a process called <u>Replication</u> using <u>DNA Polymerase</u>. The DNA ----> RNA is a process called <u>Transcription</u> using <u>RNA</u> <u>Polymerase</u>. The RNA ----> Protein is a process called <u>Translation</u> using <u>amino-</u> <u>acyl tRNA synthetases</u> and <u>ribosomes</u>.

18	(3')UAGCAUGGCAAU(5'). Also acceptable is (5')UAACGGUACGAU(3').
10	
20	Answers would include: initiation of transcription, post- transcriptional processing (differential splicing, poly-adenylation, capping, mRNA degradation), differential transport from the nucleus to the cytoplasm, initiation of translation, post-translational processing (including protein degradation).
0.1	Reverse transcriptase is used to make first a single-stranded DNA complementary to mRNA, then a double-stranded DNA.
21	They are comparable in that they both are used to MAKE MORE. They both depend on replication of DNA as well They contrast in that one is invivo and the other is invitro; one is laborious and one is simple; one requires previous knowledge of DNA sequences and the other does not.
	The 2'-OH group in RNA acts as an intramolecular nucleophile, which is activated for catalysis by alkali, for attack on the phosphate in the phosphodiester bond. In the hydrolysis of RNA a 2'-3'-cyclic intermediate is formed. DNA does not have a 2'-OH group.
23	
	GenBank data is in the form of DNA sequences. Protein data is in the form of protein structures with a list of $x$ , $y$ , $z$ coordinates.
24 25	Base stacking interactions are higher for G/C base pairs.
	<ol> <li>a) the 5'-&gt;3' <u>polymerase</u> activity of DNA Pol III; 10<sup>-4</sup> to 10<sup>-5</sup></li> <li>b) plus the 3'-&gt;5' <u>exonuclease</u> activity of DNA Pol III; 10<sup>-4</sup> to 10<sup>-5</sup></li> <li>the 5'-&gt;3' <u>polymerase</u> activity of RNA polymerase; 10<sup>-4</sup></li> <li>the editing or <u>hydrolase</u> activity of some aminoacyl-tRNA <u>synthetases</u>; 10<sup>-4</sup> to 10<sup>-6</sup></li> <li>the <u>GTPase</u> activity of EF-Tu which is slow to allow correct condon-anticodon interactions; 10<sup>-4</sup></li> </ol>
26	AMAse must be a hexamer. This would account for the seven bands observed upon hybridization of the two types of subunit by incubation at 37 °C $B_6$ , $B_5L$ , $B_4L_2$ , $B_3L_3$ , $B_2L_4$ , $BL_5$ , & $L_6$ , where B stands for
0.5	the brain enzyme and L for the liver enzyme.
21	<ul> <li>(a) This substitution would decrease terminator function by <u>stabilizing</u> the RNA-DNA hybrid duplex.</li> <li>(b) These mutations would decrease terminator function by <u>destabilizing</u> hairpin formation, and the RNA-DNA hybrid will be stabilized as a result.</li> <li>(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.</li> </ul>
28	

A wax consists of a long-chain fatty acid in ester linkage with a long-chain fatty alcohol.

## No. on

- Test Correct Answer
  - 29 (a) Triacylglycerols have three fatty acyl groups in ester linkage with glycerol; they are very hydrophobic, because the ester linkages mean that the carboxyl groups cannot ionize. Phosphoglycerides have a polar region at their head group, where a phosphate in a phosphodiester linkage bears a full negative charge, and the head group (serine, ethanolamine, choline, etc.) may also be charged and is in any case (b) The phospholipid is amphipathic, having both polar and polar. nonpolar regions, and it forms lipid bilayers spontaneously in water. (c) These lipid bilayers are stabilized by the energy gained from burying hydrophobic groups out of contact with water. A hydrophobic chain in water forces the formation of a cage of immobilized water molecules around it; when several hydrophobic regions cluster in a bilayer, the surface area exposed to water decreases, and the water molecules in the cage are released, with a gain in entropy that drives the formation of the bilayer.
  - 30 Old model: freeze fracture showing proteins inside, existence of integral membrane proteins, membrane proteins with alpha-helices

Fluid Mosaic model: heterokaryons, FRAP

31 Ericamycin probably blocks translocation.

32

E A

- I
- G

Η

- F
- В
- В
- в
- D C B

a c

33

- - b f
  - d
  - е

34

d b

С

a