Name (Last, First) _____

Social Security # _____

Graduate _____ or Undergraduate _____

Biochemistry 353
Final Exam
May 15, 1998

Part I :		_____ /	32
Part II :		_____ /	12
Part III:	1.	_____ /	9
	2.	_____ /	14
Part IV:		_____ /	25
Part V :		_____ /	20
Part VI:		_____ /	10
Part VII:		_____ /	54
Part VIII		_____ /	24
Total :		_____ /	200

PART I. Multiple Choice (32 points, 2 points each). Circle all of the statements that are correct. Two points for each correct answer circled, **-1** for each incorrect answer circled, so do not guess wildly. There may be several correct answers to a question or none.

1. The genetic code was deciphered more than 30 years ago through combined efforts in organic synthesis and in enzyme isolation.

a. AUC is the most commonly used initiation codon in prokaryotes and eukaryotes.
b. The genetic code is universal, so human cDNAs will always be recognized and expressed in bacteria.
√ c. The genetic code is degenerate and non-overlapping at the same reading frame.
d. UAA, UAG, and UGC are three commonly used termination codons.
e. Polynucleotide phosphorylase uses ribonucleoside triphosphate as activated substrates and does not rely on a DNA template for RNA synthesis.

2. Recombinant DNA technology is facilitated by the discovery of enzymes that bind DNA and the development of cloning vectors.

a. The DNA ends generated by Bam HI and Bgl II are compatible. Thus, Bam HI and Bgl II are defined as isoschizomers.
√ b. T4 DNA polymerase can be used to convert a 3' overhang to a blunt end.
c. Most commonly used enzymes for molecular cloning are type III restriction enzymes.
d. An essential feature of a cosmid is the lambda attachment sites which facilitate the propagation of a cosmid in bacteria.
e. Centromeres are essential, whereas telomeres are dispensible, in designing a yeast artificial chromosome for cloning a large DNA fragment.

3. DNA replication is a highly coordinated event that requires many protein factors.

a. PCNA enhances the processivity of DNA polymerase α in eukaryotic DNA replication.
b. Klenow enzyme is often used in nick translation to prepare ^{32}P-labeled DNA fragments.
c. AZT is a compound that inhibits the activity of HIV integrase.
√ d. G-quartets may form at the ends of chromosomes and advance the aging process.
√ e. Two β subunits of the bacterial DNA polymerase III holoenzyme are required to form a DNA clamp on each DNA strand.

4. DNA repair is essential for correcting the damage occurring on DNA molecules.

√ a. DNA photolyase can bind and also cleave thymine dimers induced by UV light.
b. A 14-nucleotide DNA fragment is released by uvrABC endonuclease during the mismatch repair process.
c. MutS, MutL, and MutH are involved in the nucleotide excision repair in E. coli.
d. Recombinational repair can occur either before or after DNA replication.
√ e. Binding of RecA to single-stranded DNA stimulates LexA self-cleavage during the SOS response.

5. RNA processing is a posttranscriptional modification that is required to make RNA molecules fully functional.

a. U2 snRNP binds the 5' splice site to initiate the splicing of mRNA precursors.
b. A 7-methyl cytosine is often added to the 5' end of the mRNA precursor to protect the RNA molecules from being degraded by phosphatases and ribonucleases.
√ c. CstF enhances the binding affinity of CPSF to the AAUAAA polyadenylation signal.
d. The A residue in the branch point of the lariat intermediate during mRNA precursor splicing is joined to two nucleodies.
e. Primase is the enzyme that adds a stretch of A residues at the 3' end of RNA.

6. Transcription is a process that transmits the genetic information from DNA to RNA.

a. α-amanitin can inhibit the function of both RNA polymerase I and II, but not RNA polymerase III.
√ b. An A/T-rich sequence often found in bacterial promoters is recognized by the σ subunit of prokaryotic RNA polymerase.
c. In eukaryotes, RNA polymerase I is responsible for the synthesis of transfer RNA.
d. TFIIB is the initiation factor that recognizes the A/T-rich region often found in the eukaryotic promoters.
e. Rho protein is the bacterial termination factor which is required to terminate all bacterial transcription.

7. Lambda (λ) phage has two distinct life cycles in an infected bacterial cell.

a. DNA damage induces the switch from the lytic to the lysogenic life cycle.
√ b. Only the cI gene is expressed during the maintenance stage of the lysogenic cycle.
c. Cro protein has higher affinity to O_L1 and lower affinity to O_R3, following the order $O_R3 < O_R2 < O_L3 < O_L2 < O_R1 < O_L1$.
d. Lysogeny is terminated first by proteolysis of Cro protein.
e. The lytic pathway requires two protein factors. N turns on the expression of immediate-early genes, whereas Q affects the expression of delayed-early genes.

8. Prokaryotic transcription is controlled by many sequence-specific DNA-binding proteins.

√ a. A chimera protein containing an N-terminal α-helix from phage 434 and a C-terminal α-helix from phage P22 will bind to the P22 operator.
b. Inducible catabolic operons are globally regulated by CAP protein containing bound cyclic GMP.
c. Binding of Lac repressor to the Lac operator inhibits the promoter recognition by RNA polymerase due to steric interference.
d. The C protein encoded by the *araC* gene is a transcriptional activator which can be converted into a repressor after binding to L-Arabinose.
√ e. Attenuation of the amino acid-synthesizing operons is mediated by the tight coupling of transcription and translation and is only found in prokaryotes.

9. The structures of several DNA-binding domains have been solved.

a. The Cys_2-His_2 zinc-finger DNA-binding domains are very common in the nuclear hormone receptor superfamily.
√ b. The helix-turn-helix and β ribbon are two commonly found motifs in prokaryotes.
c. The helix-loop-helix motif is sufficient for dimerization and DNA-binding.
d. Homeodomains are structurally similar to the helix-loop-helix DNA-binding domain.
e. TBP is a major groove-binding protein which bends DNA after its binding.

10. General transcription factors are accessory proteins required for accurate initiation by eukaryotic RNA polymerases.

a. The snRNA genes are transcribed by RNA polymerase III and contain intragenic promoter elements.
√ b. TFIIA mainly acts as an antirepressor to overcome the repressive functions of negative factors.
√ c. TFIIF is the basal transcription factor that travels with RNA polymerase II during the elongation step.
d. The TATA box is a core promoter element only found in the class II genes transcribed by RNA polymerase II.
e. UBF is a general transcription factor required for transcription by RNA polymerase III.

11. Chromatin is a unique structure found in eukaryotes due to protein-DNA interaction.

a. DNA enters and leaves a histone octamer at the opposite side of a nucleosome.
b. Histones are acidic proteins that bind DNA to form a higher order structure.
√ c. H4 is the most conserved histone, whereas H1 is more divergent through evolution.
d. The length of the linker DNA remains constant through evolution.
√ e. Histone acetyltransferase and histone deacetylase both regulate the acetylation state of histones which is important in modulating gene activity.

PART II. Fill-in (12 points, 1 point each). Write in the blank the word or phrase that best completes the statement.

1. The shift of a hydrogen atom in a DNA base between the keto and enol forms is defined as ____**tautomerization**_____.

2. The helical winding of the DNA strands around each other is defined as ___**twist**___, whereas ___**writhe**___ is a measure of the coiling of the axis of the double helix.

3. __**Aphidicolin**_ is used to inhibit DNA synthesis and is also used to synchronize eukaryotic cells at the G1 phase.

4. The __**Ames**___ test is a screening procedure to identify potential mutagens using Salmonella as a tester strain.

5. _____**Guide**____ RNAs contain 3' oligo(U) tails which are used to provide U residues during RNA editing which is carried out by a protein complex called ___**editosome**__.

6. The group I self-splicing intron requires a ____**guanosine**_____ cofactor to initiate the transesterification reaction.

7. Most bacterial operons specifying the expression of enzymes involved in the metabolism of lactose, arabinose, and galactose were not induced in the presence of glucose. This phenomenon is known as _____**catabolite repression**_____.

8. _____**Orphan**_____ receptors are those nuclear hormone receptors with no ligands identified when the receptors were initially isolated.

9. ___**Micrococcal nuclease**___ is an enzyme that releases individual nucleosomes after complete digestion.

10. A _____**spooling**_____ model explains how RNA polymerase can transcribe through a chromatin template without dissociating the histone octamer from the DNA template.

PART III. Short Answers (credit as specified).

1. (9 points) The substitution of C to U can be found occasionally in DNA and RNA molecules. Briefly describe three ways to generate this substitution.

1) by a spontaneous reaction due to oxidative deamination

2) by chemical mutagens such as nitrous acid

3) by an RNA-editing enzyme called cytidine deaminase

2. (14 points) A lot of proteins required for *E. coli* DNA replication are well characterized. Please list seven bacterial proteins involved in the initiation of prokaryotic DNA replication, and describe in a few words the role of each enzyme in bacterial DNA replication.

Protein	Role
DNA gyrase	**introduces negative supercoils**
Helicase	**unwinds the double helix**
SSB	**stabilizes single-stranded regions**
Primase	**synthesizes RNA primers**
DNA polymerase III holoenzyme	**synthesizes DNA**
DNA polymerase I	**erases primer and fills gaps**
DNA ligase	**joins the ends of DNA**

Part IV True / false section: circle appropriate letter (25 points; 1 point each)

1. T / **F** The energy for driving amino acylation of a tRNA comes from hydrolysis of GTP.

2. T/ **F** The primary function of the small ribosomal subunit is to catalyze peptide bond formation, whereas the large subunit binds mRNA and tRNA.

3. T / **F** A mutagen is a compound which alters membrane structure.

4. **T** / F A carcinogen is an agent which causes cancer.

5. **T** / F Ser-linked GlcNAc is a sugar modification found on some cytoslic proteins, and is thought to function analogously to phosphorylation.

6. T / **F** Endoplasmic reticulum bound ribosomes have a subunit not found in soluble ribosomes.

7. T / **F** Lysosomal proteases require ATP to cleave peptide bonds.

8. **T** / F Isopeptide bonds are found in bacterial cell walls and in ubiquitinated proteins.

9. T / **F** GTP is used to help unfold proteins prior to insertion into the proteosome for degradation.

10 **T** / F Some N-linked sugar modification reactions occur in the cytosol of the cell.

11. T / **F** Clathrin coated vesicles remain coated from donor to acceptor membrane.

12. **T** / F The electric organ of torpedo fish is notable for it's usefulness as a source of proteins involved in synapse function.

13. **T** / F SNARE proteins involved in membrane fusion events are the targets of botulinus toxin produced by bacteria.

14. T / **F** Unlike protein and nucleic acid synthesis, sugar chains on proteins are heterogeneous because sugar encoding genes are recombined somatically.

15. T / **F** Proteolytic activities within the proteosome are maximal due to maintenance of a low internal pH.

16. T / **F** The integration of a retroviral genome into the host chromosome involves attack of the 3' OH group of the viral DNA upon the ribose moiety of the chromosomal double helix.

17. **T** / F Viral envelopes can contain membrane derived form the host cell plasma membrane.

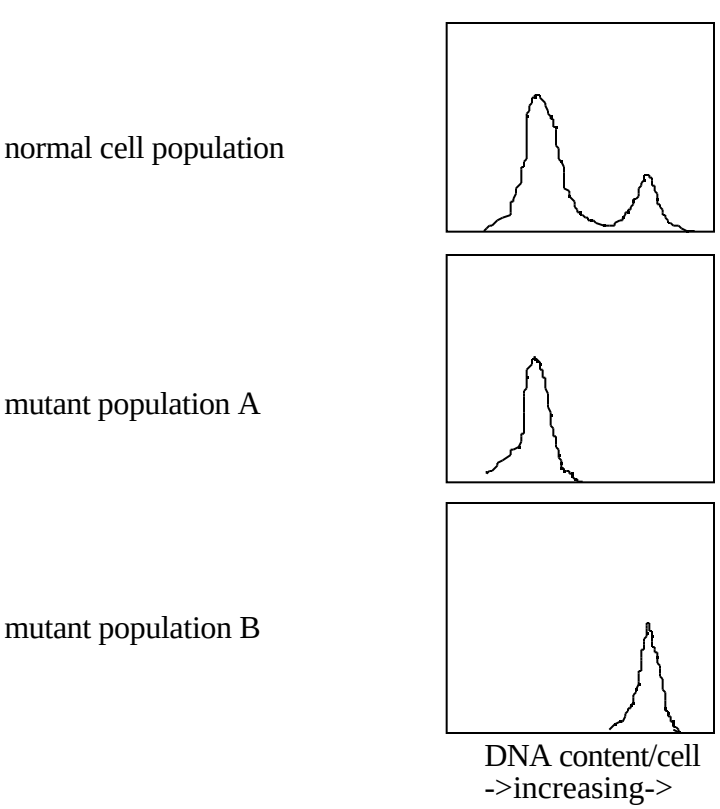
18. **T** / F Proteoglycans have their sugar chains attached in Serine-Glycine (Ser-Gly; S-G) repeat sequences which are flanked by acidic residues.

19. T / **F** The GTP bound form of Ran has a high affinity for importin $\alpha\beta$.

20. T / **F** apoptosis involves the transcriptional activation of pro-apoptotic genes necessary to carry out the cell death program.

21. T / **F** Mucins contain a high content of carboxylated and highly sulfated sugars.

The following are FACS (flow cytometry) histograms of numbers of cells versus chromosomal DNA content per cell (amount of DNA each cell has). Use these to answer T /F questions 22-25:



22. **T** / F Cells in mutant population A could have a defective start kinase.

23. T / **F** Cells in mutant population A could have a defective mitotic cyclin.

24. T / **F** Cells in mutant population B could have a defective start kinase.

25. **T** / F Cells in mutant population B could have a defect in MPF phosphorylation.

Part V Fill in the blank section (20 points; 1 point for each blank)

1. Heterotrimeric G-proteins associate with membranes because the ____***alpha***_____ and ____***gamma***_____ subunits are ____***lipid modified***_____ (feature of the molecules).

2. The active site residue of caspases is ____***cysteine***_____ and these enzymes cleave after _____***aspartate***_____ residues.

3. Mutating a Tyrosine to a ___***Phe (phenylalanine, F)***__ (amino acid residue) would be the best way to test the role of the tyrosine as a potentially important target for phosphorylation.

4. ***dimerization (multimerization)***___ is the simplest and most often used mechanism of activating a transmembrane tyrosine kinase receptor, and often results in autophosphorylation.

5. _____***LTRs***_____and_____***packaging signal***_____ are the two cis acting elements which must be present on a retroviral vector carrying a therapeutic gene for gene therapy (assuming an appropriate packaging cell line exists).

6. _____***GAG***____ , _____***POL***_____and _____***ENV***_____ are the three genes that must be expressed in a packaging cell line (i.e. supplied in trans) to make replication defective viral particles for gene therapy (using a retrovirus vector).

7. Ion channels in the plasma membrane of a neuron open to create the action potential. _____**Na+**_____ ions flow _____**in**_____ (direction) to give rise to the rising phase of the action potential (depolarization), and _____**K+**_____ ions flow _____**out**_____ (direction) to assist in the falling phase (repolarization).

8. multiple sclerosis results in deficiency in action potential propagation as a result of autoimmunity against a part of the nervous system (antibodies recognize and destroy parts of the nervous system). _____***Myelin***_____ is the target of the antibodies.

9. G-proteins are notoriously poor enzymes. They possess a weak ____***GTPase***_____ activity (enzymatic activity). Enzymatic activity occurs at an appreciable rate due to the activity of __***GTPase activating proteins (GAPs)***___.

10. The common theme seen in translocation of proteins across the ER membrane, across the mitochondrial membrane, across the chloroplast membrane, and into the proteosome is that proteins must be _____***unfolded***_____ to pass.

Part VI Matching section (10 points)
The following questions are about modular protein-protein interaction domains. Place the letter corresponding to the following next to the appropriate descriptions below. Some descriptions may get more than one letter, some letters may be used more than once **(-1/2 point for each incorrectly placed letter: make only good guesses, 10 points maximum, 0 points minimum)**

A) PDZ domain
B) SH2 domain
C) SH3 domain
D) PTB domain

_____**B,D**_____binds phosphorylated tyrosines

_____**C**_____binds proline rich motifs in proteins

_____**A**_____binds Glu(Ser/Thr)AspVal-COOH (i.e. C-terminal E(S/T)DV)

_____**A,D**_____ uses anti-parallel β-sheet interaction for binding

_____**B**_____uses "two-pronged-plug" for interaction

_____**C**_____uses mainly hydrophobic interactions for binding

_____**D**_____reads out amino acids amino terminal to TyrPO4

_____**B**_____reads out amino acids carboxyl-terminal to TyrPO4

Part VII short answer: use only the space provided, single words or phrases are sufficient (54 points total, points for each question indicated in ())

1. (2) Addition of a farnesyl group to a protein is insufficient for membrane attachment. List two other protein modification commonly found on farnesylated proteins which enhance their affinity for membranes.
methylation
palmitoylation

2.(4) What are two steps in protein synthesis that sometimes result in GTP hydrolysis which is non-stoichiometric with polypeptide chain elongation?

hydrolytic site activity in AA-tRNA synthetase
EF-Tu delivery of aminoacyl-tRNA into A site if wrong AA is delivered

3. (2) The stomach is an organ compartmentalized proteolytic system, the lysosome is an organellar compartmentalized proteolytic system. What is the third method of compartmentalizing proteolysis (give the name of the structure)?

proteosome

4.(2) What two experimental tools (chemical reagents, not a visualization device) allowed investigators to conclude that there is a continuous aqueous channel from the P-site in the ribosome all the way through the translocon?
fluorescently labeled amino acids
water soluble fluorescence quenchers

5.(2) What are the two sources of degeneracy in the genetic code?

wobble base pairing
multiple codons (tRNAs) for some amino acids

6.(6) Name the three species of RNA involved in translation and briefly (1-5 words) give their function

mRNA: informational
tRNA: adapter / charged intermediate
rRNA: structural / catalytic

7. (2) tRNA charging is similar to fatty acid activation. What is the source of energy for these reaction, and what drives these reactions forward (makes the reactions irreversible)?

ATP ; PPi hydrolysis by pyrophosphatase

8.(4) what are two differences between endocytosis of the transferrin receptor and the EGF receptor?
(2)constitutive versus ligand induced endocytosis
transferrin is constitutively cycled while EGF receptor is only internalized following ligand binding to the receptor.
(2)The transferrin receptor is recycled while the EGF receptor is degraded.

9.(2) Signal transduction is often characterized by cascades of enzymes (i.e. KinaseKinaseKinase-> KinaseKinase->Kinase or ProteaseProteaseProtease->ProteaseProtease->Protease). What are two advantages (properties)of these kinds of systems?
(1)amplification (must have)
(1)more opportunity for regulation or integration of pathways

10.(2) adrenaline (also called epinepherine) can activate cAMP synthesis in some cells but inhibits cAMP synthesis in other cells. How can this happen?
the cells have different receptors which interact with different G proteins. The different G-protein have different effects on adenylyl cyclase.

11.(2) Pepsin activation by proteolysis is independent of pepsin concentration. What does this suggest about the mechanism of proteolysis?

intramolecular reaction

12.(2) give one example of a substrate which is activated by cleavage of a caspase and one example of a substrate which inactivated by a caspase cleavage.
ICAD is inactivated by cleavage
nuclear lamin function is inactivated by cleavage
other caspases are activated by caspase cleavage
gelsolin is activated by cleavage

13.(4) The transmembrane tyrosine kinase receptor to ras activation pathway is highly conserved through evolution. What are the conserved proteins in between the receptor and ras, and what are the functions of these proteins?
adapter (Grb2/sem5/drk) from receptor to GEF
GEF guanine nucleotide exchange factor for ras exchanges GTP for GDP to activate ras

14.(2) define ex vivo gene therapy

introduction of therapeutic gene into cells removed from patient followed by re-implantation into patient

15.(2) What is the function of SH2 domains in STATs ?

SH2 domains mediate dimerization by binding phosphorylated tyrosines on other STATs.

16.(2) What is the consequence of having TGFbeta activation transcribe genes encoding inhibitory smads?

turn off it's own signal (auto-regulatory negative feedback)

17.(2) proteins which compose the cytoskeletal filaments which support cells share a common interaction theme which enables them to fulfill there functional role. What is this property?

Homomultimerization: homopolymerize into filaments

18.(5) A major contributor to the transmembrane potential across the plasma membrane of cells (including neurons) is due to an electrogenic, ion pumping, ATPase. What ions are pumped, which direction are they pumped and what makes this electrogenic?

Na+ out, K+ in; more Na+ than K+ makes a charge differential

19.(2) Rous in 1911 injected a cell free extract from chicken sarcomas into other chickens and found that the injected chickens got sarcomas. What was the significance of this experiment?

viruses (1)could cause cancer (1).

20.(3) Some oncogenes have been identified in the absence of a viral version of the protein which are mutated. List three means of generating an oncogene from a proto-oncogene which don't involve a virally encoded mutant version of the gene.
point mutation activating an oncogene
insertional mutagenesis altering the function of expression of a proto-oncogene
chromosomal rearrangements resulting in the altered function or expression
amplification of a chromosomal locus

Part VIII short essay: use only the space provided. Flow diagrams will work for question 2, 3, and 4 (24 points total, points for each question indicated in ())

1.(4) The protein inaD is involved in visual signal transduction in drosophila photoreceptor cells. This protein is composed of 5 PDZ domains. What do these domains do (in a general sense), and what is the advantage of this arrangement for visual signal transduction?
bind molecules involved in the signal transduction cascade (act as a scaffold)
physical proximity maximizes speed and fidelity (minimizing potential cross-talk)

2.(8) You have a small molecule ligand which activates a Heterotrimeic G-protein containing Gsα. List or draw a schematic of the flow of protein-protein interactions from ligand receptor binding to protein kinase A (PKA or A kinase) activation.

ligand + receptor changes receptor
ligand receptor complex catalyzes binding of GTP to Galpha and release of GDP
Galpha-GTP releases Gbeta-gamma
free Galpha-GTP stimulates adenylyl cyclase->cAMP is produced
cAMP binds regulatory subunits of PKA
regulatory subunits dissociate from PKA catalytic subunit
catalytic subunit is now free active kinase

3.(6) What are the two protein components of Maturation Promoting Factor (MPF; also called Mitosis Promoting Factor) and what three enzymatic activities are needed for activation of MPF? What process terminates MPF function? (you may make a list, or draw a labeled diagram)

cyclin dependent kinase (cdc2 or cdk or p34 OK)
cyclin (mitotic cyclin better)
Thr kinase
Tyr Kinase
phosphatase (Tyrosine phosphatase)
cyclin destruction by ubiquitin/proteosome pathway

4.(6) What two second messenger molecules collaborate to activate protein kinase C, and what is the source of these molecules (how are they generated, where do they come from)?

1. Ca2+ released from intracellular stores (ER) by action of IP3 which is released from PIP2 by phospholipase C
2. DAG released from PIP2 by action of phospholipase C