Name_____

Name (Last, First) _____

Graduate _____ or Undergraduate _____

Biochemistry 353, Spring 2002 Final Exam, May 4, 2002

Before you start, PRINT your name on the top of this page, and indicate graduate or undergraduate. Be sure to print your name on the top of each 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 answer in the SAME amount of space on the back of the page. However, you MUST indicate on the FRONT PAGE of the exam which questions have been answered on the back of a page or they will NOT be graded.

The exam has 14 numbered pages. Check to make sure that you have all of the pages.

Part I :	_____ /	24
Part II :	_____ /	24
Part III :	_____ /	20
Part IV :	_____ /	10
Part V :	_____ /	24
Part VI :	_____ /	19
Part VII:	_____ /	79
Total :	_____ /	200

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Part IV True / false section: circle appropriate letter (10 points; 1 point each)

1. T / F Nuclear import occurs by a process of vesicular transport.

2. T / F SNARE proteins involved in membrane fusion events are the targets of cholera toxin produced by bacteria.

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

4. T / F All of the carbon atoms in cholesterol are derived from acetate.

5. T / F G-protein coupled receptors are guanine nucleotide exchange factors for heterotrimeric G proteins.

6. T / F Inactivating a positive regulator of the cell cycle results in abnormally large cells.

7. T / F The translocon creates a pore through the endoplasmic reticulum (ER) membrane which allows N-linked core carbohydrates to flip across the membrane from their site of synthesis in the cytoplasm to the site of attachment to proteins in the lumen of the ER.

8. T / F Signal recognition particle (SRP) binding to the signal sequence of a secreted protein arrests translation and targets the protein to the plasma membrane for secretion.

9. T / F Because the primary amino acid sequence of a membrane protein determines its topology in the membrane, detergent solubilized membrane proteins incorporated into lipid bilayers in vitro adopt their correct orientation.

10. T / F Acetate and Choline are the substrates used but choline-acetyl-transferase to synthesize acetylcholine.

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Part V Fill in the blank section (24 points; 1 point for each blank)

1. _____ and_____ are the two protein components of Maturation Promoting Factor (MPF; also called Mitosis Promoting Factor). _____ ,and _____ , and _____ are the three enzymatic activities needed for activation of MPF, while termination of MPF function involves _____.

2. _____ is the protein synthesis inhibitor which was instrumental in developing the concept of unique sites in the ribosome. In the absence of translocation this inhibitor can be transferred to _____ but not_____.

3. Mutating a _____(amino acid residue; single letter code) to a _____ (amino acid residue; single letter code) would be the best way to test the role of a tyrosine as a potentially important target for phosphorylation.

4. _____ is the GEF (guanine nucleotide exchange factor) for EF-Tu.

5. The active site residue of caspases is _____ and these enzymes cleave after _____ residues.

6. Small G-proteins are notoriously poor enzymes. They possess a weak _____ activity (enzymatic activity). Enzymatic activity occurs at an appreciable rate due to the activity of _____.

7. _____ and _____ and _____ are the three genes common to all retroviruses.

8. _____ or_____ plus _____ are the two mechanisms used by a T-Cell to initiate apoptosis in a target cell.

9. _____ and _____ are the cofactors required to generate an active complex containing Apaf which stimulates apoptosis.

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Part VI short answer: use only the space provided, single words or phrases are sufficient (19 points total, points for each question indicated in ())

1.(3) What are two steps in protein synthesis that sometimes result in GTP hydrolysis which is non-stoichiometric with polypeptide chain elongation, and what is gained by this extra expenditure of energy?

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

3.(3) Signal transduction is often characterized by cascades of enzymes (i.e. KinaseKinaseKinase-> KinaseKinase->Kinase or ProteaseProteaseProtease->ProteaseProtease->Protease). What are three advantages (properties) of these kinds of systems?

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

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5.(2) Acetylcholine can alter cAMP levels in some neurons but in other neurons it causes changes in plasma membrane ionic permeability. How can a single neurotransmitter do different things to different cells?

6.(4) Binding of the G alpha subunit of a heterotrimeric G protein to GTP is partially mediated by switch regions I, II, III of the G alpha protein. What protein-protein interactions are also mediated (at least in part) by these regions of the G alpha subunit, and in which nucleotide bound states do these interactions occur?

Part VII essay: use only the space provided. (79 points total, points for each question indicated in (); <u>points may be deducted for incorrect statements;</u> please take your time and think before you write). As always brevity is a virtue, many of the questions can be answered with key terms and phrases.

1.(6) How do changes in pH facilitate the normal cycling of transferrin though the endocytic pathway (where do these changes occur, and what is the outcome of the pH change)?

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2.(5) Snare proteins mediate a critical aspect of intracellular membrane fusion events in eucaryotic cells and are thought to function analogously to the HA fusion protein which mediates fusion of viral membrane with host cell membrane. What are the major differences in the organization, and means of control, of these fusogenic molecules/complexes?

3.(5) Diisopropylphosphofluoridate (DIPF) inactivates serine proteases, and its analogue Sarin (a nerve gas) inactivates the catalytically similar enzyme Acetylcholinesterase (AChE). Bovine pancreatic trypsin inhibitor (BPTI) and α1-antitrypsin are also effective in inhibiting serine proteases (trypsin and elastase respectively), but do so by a mechanism which is from the mechanism employed by DIPF/sarin. Please describe the general mechanism of action of these two classes of inhibitors.

4. (6) Describe the linkages (protein-lipid linkage chemistry and location in protein) used in generating fatty acylated (two types) and prenylated (one type) proteins.

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5.(6) Compare and contrast the carbohydrate present on a proteoglycan with those present on a protein with N-linked carbohydrate. Please limit you answer to the defining characteristic of the sugars (general organization and important modifications, not the specific sugars), and the sugar-protein linkage.

6.(8) Signal transducing receptor systems can consist of two different receptor molecules, one of which is ligand binding the other of which generates the signal. One signal enhancing property that derives from this arrangement is due to a reduction in the degrees of freedom experienced by the ligand when it is bound to the ligand binding receptor for subsequent presentation to the signaling receptor. What is meant by reduced degrees of freedom and explain the relevant reductions.

7.(6) The ribosome, the nucleosome and viruses are three nucleoprotein particles that were discussed in class. Briefly describe the relationship between the nucleic acid and the protein components (general organization) of these three nucleoprotein particles.

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8.(7) Describe the features (types of amino acids, general secondary structure if relevant, and location in the protein) of the signals which direct proteins to the 1) mitochondria, 2) lysosome, and 3) nucleus.

9.(8) SREBP is a membrane bound transcription faction which is sensitive to cholesterol concentration. Describe the major steps used in regulating this transcription factor in response to cholesterol levels.

10. What effect upon PKA activity would you expect if you

a) (2) mutated the psuedosubstrate sequence of the PKA regulatory subunit so that it could not bind the catalytic subunit of PKA?

b) (2) mutated the cAMP binding sites of the PKA regulatory subunit so that they could not bind cAMP?

c) (2) Which, if either, of the above mutants would act as a dominant negative if overexpressed in the presence of the wild type versions of PKA subunits (assume that it is NOT possible to express the mutants at levels which can interfere with cytosolic cAMP concentrations)?

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11.(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.

12.(8) Receptor tyrosine kinase activation can result in phosphorylation of transcription factors and changes in gene expression. Cytokine receptor activation can also alter gene expression. In general terms, compare and contrast the signaling induced by these two classes of receptors, and identify which of these mechanisms is similar to the mechanism employed by TGF beta type ligands which signal through Ser/Thr transmembrane kinases.