Name (**Last, First**)_____

Graduate _____ or Undergraduate _____

Biochemistry 353

Third hourly Test

April 8, 2002

Part I :	_____	/ 14
Part II :	_____	/ 24
Part III :	_____	/ 20
Part IV :	_____	/ 28
Part V :	_____	/ 14
Total :	_____	/ 100

Part I True / false section: circle appropriate letter (1point each: 14 points total)

1. T / F During protein synthesis, non-stoichiometric hydrolysis of GTP can occur during the tRNA charging step, while non-stoichiometric hydrolysis of ATP can occur during the elongation step.

2. T / F The shine dalgarno sequence is found downstream of the initiator AUG in bacterial mRNAs and base pairs with the 16S ribosomal RNA.

3. T / F An intermediate in the formation of charged tRNA is a mixed anhydride species.

4. T / F Non-stoichiometric hydrolysis of GTP and ATP during protein synthesis can enhance the speed of protein synthesis.

5. T / F If an organism has only a single tRNA for the amino acid tyrosine this tRNA could be mutated in its anticodon to yield a viable frameshift suppressor.

6. T / F Selectins are proteins found on lymphocytes which allow specific recognition of sugar residues on endothelial heparin sulfated proteoglycans (HSPGs).

7. T / F Some O-linked sugars are linear unbranched polymer of repeating disaccharides.

8. T / F Tunicamycin is a structural analog of UDP GlcNAc and inhibits the synthesis of dolichol-PP-GlcNAc resulting in a block of carbohydrate addition to asparagine residues on proteins.

9. T / F Asn-linked GlcNAc is a sugar found on some cytosolic proteins, and is thought to function analogously to phosphorylation.

10. T / F The viscosity of mucous derives from the highly charged nature of proteoglycan sugars.

11. T / F I-cell disease is a lysosomal storage disease characterized by a lack of lysosomal proteases in the lysosome due to a defective phosphotransferase activity.

12. T / F Glycoprotein glucosylation is a sugar modification which can keep a protein from leaving the ER.

13. T / F Aberrant proteolysis is associated with in Alzheimer's disease.

14. T / F Fusion of eucaryotic intracellular membranes (e.g. fusion of a golgi derived vesicle with the plasma membrane) involve SNARE proteins which undergo pH dependent conformational changes.

Part II Fill in the blank section (24 points; 1 points each blank)

1. The ribosome has three functionally distinct sites (A, P, and E). _____ is the only charged tRNA which can enter the P site, all other charged tRNAs enter the A site. Experiments with _____ allowed this distinction.

2. Polypeptide elongation during protein synthesis involves nucleophilic attack by the _____ group of the charged amino acid in the _____ site of the ribosome upon the _____ group of the polypeptide attached to a tRNA in the _____site of the ribosome.

3. Termination of protein synthesis is accomplished by transfer of the polypeptide to __ _____.

4. During protein synthesis, mRNA is read in the_____ prime to _____ prime direction.

5. Tertiary base pairing interactions between the _____ (arm / loop) and the _____(arm / loop) help to organize the three dimesional L shape of the tRNA molecule.

6. Proteins are synthesized from _____ to _____ termini.

7. Palmitoylation of proteins involves a labile _____ linkage between palmitate and _____ (an amino acid) in proteins, while myristoylation involves a stable _____linkage between myristate and _____ (an amino acid) at the _____ terminus of proteins.

8. Isopeptide bonds are found in ubiquitinated proteins between the _____ functional group of ubiquitin and the _____ functional group of _____ residues in the tagged protein.

9. Fatty acylation and prenylation are lipid modification of proteins which serve to add a hydrophobic (membrane anchoring) sequence to proteins. _____ and _____of the hydrocarbon chain are two features of the added lipid that affect the membrane affinity conferred by these modifications, while _____ is a modification of the protein backbone which accompanies prenylation that enhances membrane affinity.

Part III Matching section (20 points)

The following questions are about protein targeting to subcellular destinations and the signal sequences involved. 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 point for each correctly placed letter, -1/2 point for each incorrectly placed letter: make only good guesses, 20 points maximum, 0 points minimum)**

a. ER targeting signal sequence
b. mitochondria targeting sequence
c. chloroplast lumen targeting sequence
d. chloroplast stromal targeting sequence
e. nuclear import signal
f. Nuclear export signal
g. lysosomal enzyme targeting signal

_____ consists of a short stretch rich in basic residues

_____ contains a hydrophobic peptide core sequence

_____ is rich in hydroxylated residues

_____ effective targeting involves small GTP binding protein(s)

_____ effective targeting and transport requires heat shock proteins

_____ involves transmembrane transport of the protein

_____ involves vesicular transport of the protein

_____ this signal sequence is present at the proteins amino terminus

Part IV short answer: use only the space provided, single words or phrases are sufficient (28 points total, points for each question indicated in ())

1. (6 points) List the three types of RNA involved in protein synthesis and their function

RNA	**function**
1.	
2.	
3.	

2. You have developed a series of EF-Tu mutants which differ in their rates of ribosome stimulated GTP hydrolysis.

a) (1 point) What is the effect of using a mutant with a faster GTPase on speed of translation?

b) (1 point) What is the effect of using a mutant with a slower GTPase on fidelity?

3. (2) What are the two functions served by EF-Tu?

4. (2) Interactions between SNARE proteins on donor and acceptor membranes are involved in intracellular membrane fusion events. Relative to the actual fusion event, when will a v- and t-SNARE complex occur between the proteins in "cis", and when will a v- and t-SNARE complex occur between the proteins in "trans" (i.e. before or after the fusion event)?

5. (2) What two modifications found on glycosaminoglycans result in a highly negative charge?

6.(2) Activation of pepsin requires a decrease in pH for a proteolytic maturation event which is independent of pepsin concentration. What does the concentration independence reveal about the mechanism of activation?

7.(2) What is the common theme (aspect of protein secondary structure) seen in translocation of proteins across the ER, across the mitochondrial membrane, across the chloroplast membrane, and into the proteosome?

8.(3) What is the signal inside an endosome which triggers fusion of the influenza virus membrane and the endosome membrane and what is the effect of the signal on the viral membrane fusion protein?

9. (4) Proteins destined to get a GPI anchor have amino and carboxyl terminal signals. What is the function and fate of each of these two signals?

10. (3) Since energy is not required for proteolysis, the ATPases of the proteosome must subserve a function other than aiding catalysis of peptide bond cleavage. List three functions ascribed to the ATPases present in the 19S component of the proteosome

Part V (14 points)

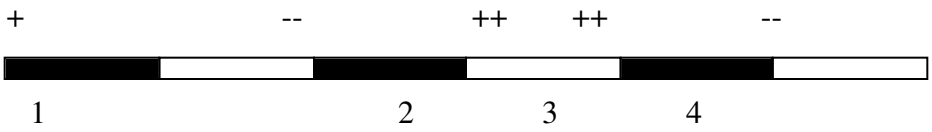
1. (6 points) You perform an experiment to look at fusion of vesicle populations. The lipids of the inner and outer leaflet of one population of vesicles get fluorescent tags at high concentration such that the fluorescence is quenched (low fluorescence), and this population of vesicles contain a V snare protein. The fluorescence will dequench (increase) if the fluorescent tag gets diluted. You mix these tagged vesicles with a second population of unlabeled vesicles (no fluorescence) which contain the cognate T snare protein. What happens to the fluorescence when you mix the vesicles under the following conditions (assume the fluorescence properties of your label (tag) has no pH dependence, and that all other components required for V and T snare mediated fusion are present)? Circle the result appropriate for each condition.

condition	fluorescence result		
neutral pH	increase	decrease	no change
basic pH	increase	decrease	no change
acidic pH	increase	decrease	no change

You repeat the experiment in the presence of dithionite, using mutant snare proteins which can only mediate hemi-fusion. What happens to the fluorescence under the following conditions? Circle the result appropriate for each condition.

condition	fluorescence result		
neutral pH	increase	decrease	no change
basic pH	increase	decrease	no change
acidic pH	increase	decrease	no change

2. (8 points) The following hypothetical membrane protein (left to right: amino to carboxyl precursor protein primary structure) has an amino terminal cleaved signal sequence and is cotranslationally inserted into the ER. Hydrophobic stretches (22 amino acids long) are black, hydrophilic stretches are white. Informative charged residues are indicated. You set up the following experiment to look at protein-protein interactions which occur during insertion of this protein into the ER membrane. You have derivatized a charged lysine tRNA with a bifunctional crosslinking agent. One of the functional groups is stably linked to the lysine, the other functional group can be activated by you, whenever you want (by light for example). You have the mRNA coding for the protein. You insert a single lysine residue (the only lysine residue in the protein) at the four places indicated (in different experiments) by performing in vitro translation and ER translocation assays with the mRNA and the derivatized tRNA.



You have done an exhaustive series of experiments crosslinking at many different time points. For each of the derivatized sites (1, 2, 3, 4 indicated below the protein schematic) specify whether you expect to find any of your translation product cross linked to each of the following components by placing a **yes** or a **no** in each box of the grid below **(+1/2 point for each correct answer, -1/2 point for each incorrect answer; 10 points maximum, 0 points minimum)**.

	1	2	3	4
core oligosaccharide transferase (N-linked: assume an NXS/T sequence is present in regions 2, 3 and 4 only)				
central region of translocon				
signal recognition particle				
large subunit of ribosome				