Name (**Last, First**)_____**KEY**_____

Graduate _____ or Undergraduate _____

Biochemistry 353

Third hourly Test

April 14, 2003

Part I : _____ / 20

Part II : _____ / 20

Part III : _____ / 38

Part IV : _____ / 4

Part V : _____ / 18

Total : _____ / 100

Part I True / false section: circle appropriate letter (1point each: 20 points total)

1. T / **F** Inosine is frequently found in the 3' position of the anticodon, and gives rise to a form of degeneracy of the genetic code because Inosine can base pair with multiple bases in the codon.

2. T / **F** RNA polymerase incorporates modified bases into tRNA during transcription of the tRNA encoding genes.

3. **T** / F If an organism has several tRNAs for the amino acid tyrosine one of these tRNAs could be mutated in its anticodon to yield a viable frameshift suppressor.

4. **T** / F The amino acid attached to a tRNA molecule plays no role in specifying where the amino acid will be incorporated into a growing polypeptide chain.

5. T / **F** The termination reaction during protein synthesis involves a transpeptidation reaction which proceeds through a covalent polypeptide-release factor intermediate.

6. T / **F** Diptheria toxin catalytically inactivates EF-G via ADP ribosylation of a posttranslationally modified histidine residue present in this bacterial elongation factor.

7. T / **F** Targeting a secreted protein to the endoplasmic reticulum requires a signal present at the amino terminus of the nascent polypeptide which is recognized by the translocon: an interaction which serves to slow translation.

8. T / **F** If two proteins are devoid of primary amines (blocked N-termini and no K residues) a homobifunctional NHS ester (NH_2 reactive agent) crosslinker could be an effective crosslinking agent to probe the interactions between these proteins.

9. T / **F** Nuclear import occurs by a process of transmembrane transport.

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

11. **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.

12. T / **F** Ran can regulate import and export of proteins from the nucleus because the GTP bound form promotes formation of import and export complexes.

13. T / **F** The characteristic feature of a nuclear export signal is the presence of a short stretch of positively charged residues (Lysine and Arginine; K and R)

14. **T** / F Some O-linked sugars are linear unbranched polymers of repeating disaccharides.

15. T / **F** Some N-linked sugars are linear unbranched polymer of repeating disaccharides.

16. T / **F** Lysosomal function does not requires ATP.

17. **T** / F NSF and SNAPs are protein factors which are generally required for all intracellular transport vesicle fusion events.

18. **T** / F SRP RNA is evolutionarily conserved. However, since bacteria translocate proteins post-translationally, the bacterial form of SRP RNA is missing the elongation arrest domain.

19. T / **F** The concentration independence of the proteolytic activation of chymotrypsin is good evidence that this maturation event occurs as an intermolecular reaction.

20. T / **F** prior to membrane fusion SNARE complexes exist in a cis configuration, while after fusion these complexes exist in a trans configuration.

Part II Fill in the blank section (20 points; 1 points each blank)

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

2. Non stoichiometric hydrolysis of ATP / GTP during protein synthesis can occur at the _____**tRNA charging**_____ step and _____**elongation**_____ step. This seemingly wasteful expenditure of energy serves to enhance the _____**fidelity**_____ of protein synthesis.

3. You have developed a series of EF-Tu mutants which differ in their rates of ribosome stimulated GTP hydrolysis. What is the effect of using a mutant with decreased rate of hydrolysis on <u>speed</u> of translation? ___***slower***____ . What is the effect of using this mutant upon <u>fidelity</u>? ___***increased***____.

4. _____**EF-Ts**_____is the Nucleotide exchange factor for EF-Tu while **the ribosome**_____ is the GTPase activating protein for EF-Tu.

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

6. Palmitoylation of proteins involves a labile _____**thioester**_____ linkage between palmitate and _____**cysteine**_____ (an amino acid) in proteins, while myristoylation involves a stable _____**amide**_____ linkage between myristate and _____**glycine**_____ (an amino acid) at the _____**amino / N**_____ terminus of proteins.

7. Isopeptide bonds are found in ubiquitinated proteins between the ___**c-terminal carboxyl**_____ functional group of ubiquitin and the ___**epsilon amino**_____ functional group of _____**lysine**_____ residues in the tagged protein.

8. The _____**shine dalgarno**_____ sequence is found upstream of the initiator AUG in bacterial mRNAs and base pairs with the 16S ribosomal RNA.

Part III short answer: use only the space provided, single words or phrases are sufficient (38 points total, points for each question indicated in ())

1.(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

unfolding
substrate recognition
deubiquitination
translocation

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

proteins must be <u>unfolded</u> to pass

3.(4) List three features of a signal sequence which are common to signal sequences directing a protein into the mitochondria and signal sequences directing a protein into the chloroplast, and the feature which distinguishes between these two signals.

rich in basic residues
rich in hydroxylated residues
deficient in acidic residues
(hydrophobic residues OK)

secondary structure (random coil versus amphipathic alpha helix)

4.(2) Which chemical group of tyrosine is commonly exploited by enzymes or binding proteins to allow discrimination between tyrosine and phenylalanine (e.g. tyrosyl tRNA synthetase binding Tyr). What is the basis of the discriminating interaction (type of bond)?

OH group (hydroxyl), ***hydrogen bonding***

5.(2) Which two steps of protein synthesis can consume GTP non-stoichiometrically with protein synthesis?

activation of AA , elongation

6.(2) Activation of pepsin (an aspartyl protease) requires a decrease in pH. What biochemical event does this trigger, and what type of bonding interaction is lost?

protonation of carboxylates
intramolecular salt bridges lost

7.(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?

amino directs targeting to and insertion into the ER, it is cleaved (or removed or degraded)
carboxyl directs GPI anchor attachment OR it serves as a temporary membrane attachment, it is cleaved (or removed or degraded)

8. (6 points total as indicated) Lipid modification of proteins serve to add a hydrophobic (membrane anchoring) sequence to proteins.

a) (2 points) What two features of the hydrocarbon chain affect the membrane affinity?

length of hydrocarbon chain and degree of saturation

b) (1 point) What modification of the protein backbone (which accompanies one form of lipidation) enhances the membrane affinity?

carboxy methylation

c) (2 points) Which two classes of lipid modification are found on proteins oriented toward the cytoplasm?

Fatty acylation (or palmitoylation or myristoylation) and prenylation (or farnesylation or geranylgeranylation)

d) (1 point)Which lipid modification is found on proteins oriented extracellularly?

GPI anchor

9.(4) Briefly describe two mechanisms of inhibiting a protease, one involving a small organic molecule and one involving a natural polypeptide (don't name an inhibitor, tell how it works).

covalent modification of active site residue
nonhydrolyzable (slowly hydrolyzable) substrate analog

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

decrease in pH causes conformational change exposing the fusion peptide

11.(3) Proteins targeted to the thylakoid lumen of the chloroplast have a signal sequence which is very much like the signal sequence which targets proteins to the E.R. membrane. How can this signal function to target the protein properly to the thylakoid and not the E.R?

The signal is masked by the amino terminal chloroplast (stromal) targeting domains (signal sequence) The lumenal targeting sequence is only revealed after a proteolytic cleavage event which occurs inside the chloroplast

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

sulfation and carboxylation

Part IV (4 points)

1.(4 points) You perform experiments 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). The fluorescence will dequench (increase) if the fluorescent tag gets diluted. You prepare a second population of vesicles which are unlabeled (no fluorescence). In separate experiments, the different populations of vesicles get one of the fusion protein(s) discussed in class incorporated into the lipids (in other words, group A vesicle have one kind of fusion protein(s) while group B have a different kind of fusion protein(s) incorporated). Given the following two sets of experimental results, which fusion protein(s) are likely to be incorporated in each case? (assume the fluorescence properties of your label (tag) has no pH dependence)

A.

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

The likely fusion protein(s) included is (are) **viral (HA, hemagglutinin)**

B.

condition	fluorescence result
neutral pH	increase
basic pH	increase
acidic pH	increase

The likely fusion protein(s) included is (are) **vSNARE and tSNARE (SNARES)**

Part V Matching section (18 points)

The following questions are about postranslational modifications of proteins. 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, 18 points maximum, 0 points minimum**)

a. farnesylation
b. geranylgeranylation
c. N-linked sugar
d. O-linked sugar
e. glycosaminoglycan
f. cytoplasmic GlcNAc
g. palmitoylation
h. GPI anchor

f, g, h readily reversible modification

a, b, f, g modification attached to cytoplasmic domains of proteins

c, d,e, h modification attached to extracellular domains of proteins

d, e, f covalently attached to serine residue of protein

a, b, g covalently attached to cysteine residue of protein

c covalently attached to asparagine residue of protein