

Name (Last, First) _____

Social Security # _____

Graduate _____ or Undergraduate _____

Biochemistry 353	Part I :	_____	/	10
Third hourly Test	Part II :	_____	/	16
April 13, 1998	Part III:	_____	/	15
	Part IV:	_____	/	41
	Part V	_____	/	18
	Total :	_____	/	100

Part I True / false section: circle appropriate letter (10 points)

1. T / F Proteolytic activities within the proteasome are maximal due to maintenance of a low internal pH.
2. T / F Some O-linked sugars are linear unbranched polymers of repeating disaccharides.
3. T / F Some N-linked sugars are linear unbranched polymer of repeating disaccharides.
4. T / F Coatamer coated (COP) vesicles remain coated from donor to acceptor membrane.
5. T / F An intermediate in the formation of charged tRNA is a mixed anhydride species.
6. T / F Asn-linked GlcNAc is a sugar found on some cytosolic proteins, and is thought to function analogously to phosphorylation.
7. T / F Lysosomal function requires ATP.
8. T / F The amino acid attached to the tRNA molecule plays a role in specifying where the amino acid will be incorporated into a growing polypeptide chain.
9. T/F In procaryotes, the tRNA synthetase which charges Met-tRNA^{fMET} recognizes only N-formyl-methionine.
10. T/F Diphtheria toxin catalytically inactivates the large subunit of the eucaryotic ribosome via an ADP ribosylation reaction

Part II Fill in the blank section (16 points; 2 points each)

1. During translation, mRNA is read in the 5' to 3' direction.
2. Proteins are synthesized from amino to carboxyl termini.
3. EF-Ts is the GEF (also referred to as GNRP, GNRF) for EF-Tu.
4. Name the sugar modification which can keep a protein from leaving the ER:

monoglucosylation or terminal glucosylation or glucosylation

5. Name the sugar modification which can direct a protein to the lysosome:

Mannose-6-PO₄

6. Experiments with the drug Puromycin lead to the distinction between the "A" and "P" sites in the ribosome.
7. The adapter AP2 links clathrin to cargo or transmembrane proteins.
8. NSF and SNAPs are factors which are generally required for all intracellular transport vesicle fusion events.

Part III Matching section (15 points)

The following questions are about lipid 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, 15 points maximum, 0 points minimum)

- a. farnesylation
- b. palmitoylation
- c. geranylgeranylation
- d. myristoylation
- e. GPI anchor

d saturated C14 hydrocarbon containing

b saturated C16 hydrocarbon containing

b thioester linkage

a, c thioether linkage

d, e amide linkage

b, d unsaturated hydrocarbon containing

a, c attached to Cys A A X COOH sequence

d amino terminal modification

a, c, e carboxyl-terminal modification

Part IV short answer: use only the space provided, single words or phrases are sufficient (41 points total, points for each question indicated in ())

1.(4) What are the two events in the transferrin endocytic pathway which use a change of pH, and what is the result of the pH change?

1. decrease of pH in endosome results in release of iron from transferrin

2. increase in pH when receptor transferrin complex gets to cell surface results in release of apotransferrin from the transferrin receptor.

2.(3) Where would you find an IRES sequence, what does IRES stand for, and what does this sequence do?

in a mRNA

Internal Ribosome Entry Site

allows eucaryotic ribosomes to initiate protein synthesis in the middle of a message (CAP independent ribosome binding)

3.(4) List the four steps of protein synthesis

activation of AA, initiation, elongation, termination

4.(3) what glycosylation reaction (and which step in the pathway) is blocked by tunicamycin and why?

N-linked glycosylation

transfer of UDP GLCNac to dolichol-phosphate

structural analog of UDP- GlcNAc

5.(2) The GTPase Ran is involved in nucleus-cytoplasmic transport. What important feature of the proteins which regulate Ran allows vectorial transport into and out of the nucleus to occur?

GAP localized to (or high concentration in) cytosol

GEF localized to (or high concentration in) nucleus

6.(3) One of the species of RNA used in translation has a characteristic L shaped tertiary structure. What is the name of this RNA? What is the conserved secondary structural feature of this RNA? How is the L-shape obtained from this secondary structure?

cloverleaf, (drawing OK)

tertiary interactions between DHU and T ψ C loop

7.(2) What is the GAP protein for EF-Tu and what does GAP stand for?

ribosome

GTPase Activating Protein

8.(2) what are the features of a nuclear localization signal?

short stretch of basic residues (or positively charged residues, or Lys and Arg)

9.(2) what effect does (GTP- S) have on the fidelity of protein synthesis? What effect does it have on the speed?

***increase fidelity
decrease speed***

10.(2) what two features of a prokaryotic mRNA direct the start site of protein synthesis?

***shin dalgarno sequence
AUG***

11.(3) Since energy is not required for proteolysis, the ATPases of the proteasome 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 proteasome

***unfolding
substrate recognition
deubiquitination
translocation***

12.(3) what are the sites (residues) of attachment between ubiquitin and a target protein, and what is this type of linkage called

***Ubiquitin COOH terminus and Lysine epsilon amino group of target
isopeptide bond***

13.(3) 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.

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

14.(2) list two protein modifications which use dolichol as an intermediate donor in their reaction

***N-linked glycosylation
GPI anchor addition***

15.(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 proteasome?

proteins must be unfolded to pass

Part V (18 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). The fluorescence will dequench (increase) if the fluorescent tag gets diluted. You mix these tagged vesicles with unlabeled vesicles (no fluorescence) which contain the viral fusion protein discussed in class. 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)? Circle the result appropriate for each condition.

condition	fluorescence result		
neutral pH	increase	decrease	<u>no change</u>
basic pH	increase	decrease	<u>no change</u>
acidic pH	<u>increase</u>	decrease	no change

You repeat the experiment in the presence of dithionite, using a mutant of the fusion protein 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	<u>no change</u>
basic pH	increase	decrease	<u>no change</u>
acidic pH	increase	decrease	<u>no change</u>

2.(12 points) The following hypothetical membrane protein is cotranslationally inserted into the ER. Hydrophobic stretches (22 amino acids long) are black, hydrophilic stretches are white. charged residues are indicated. You set up the following experiment to look at protein interactions which occur during insertion of this membrane protein into the ER. You have derivatized 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).



You have done an exhaustive series of experiments crosslinking at many different time points. For each of the sites (1, 2, 3, 4) 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 incorrect answer, +1/2 point for each correct answer; 12 points maximum, 0 points minimum).

	1	2	3	4
SRP 54				
MSF				
central region of translocon				
signal peptidase				
BiP				
large subunit of ribosome				