

Name (Last, First) _____

Social Security # (last four digits) _____

Graduate _____ or Undergraduate _____

Biochemistry 353	Part I :	_____ / 8
Third hourly Test	Part II :	_____ / 14
April 13, 1999	Part III:	_____ / 13
	Part IV:	_____ / 39
	Part V	_____ / 26
	Total :	_____ / 100

Part I True / false section: circle appropriate letter (1point each: 8 points total)

1. T / F Ribosomal association with endoplasmic reticulum membranes during membrane protein synthesis is mediated by the mRNA.
2. T / F A charged tRNA has an amino acid covalently linked to the 3' hydroxyl of the tRNA.
3. T / F Tertiary base pairing interactions between the "variable loop" and the "T C loop" of the tRNA molecule contribute to tRNA tertiary structure.
4. T / F Tertiary base pairing between the "T C loop" and the "DHU loop" of the tRNA molecule contribute to tRNA tertiary structure.
5. T / F Nuclear import occurs by a process of transmembrane transport.
6. T / F Fusion of a viral particle with a host cells endocytic vesicle membrane involves a pH change which drives a conformational change of the viral fusion protein.
7. 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.
8. T / F An IRES sequence in mRNA allows eucaryotic protein synthesis to begin with initial recognition of the mRNA by the ribosome occurring in the middle of the mRNA.

Part II Fill in the blank section (14 points; 1 points each blank)

1. The informational species of RNA involved in translation is messenger.
The adapter / high energy intermediate species of RNA involved in translation is transfer.
The structural and catalytic species of RNA involved in translation is ribosomal.
2. 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 increased rate of hydrolysis on speed of translation? faster. What is the effect of using a mutant with a slower GTPase on fidelity? increased.
3. Polypeptide elongation during protein synthesis involves nucleophilic attack by the amino group of the charged amino acid in the A site of the ribosome upon the ester carbon /carboxy group of the polypeptide attached to a tRNA in the P site of the ribosome.
4. Termination of protein synthesis is accomplished by transfer of the polypeptide to water.
5. SRP RNA is evolutionarily conserved. However, since bacteria translocate proteins post-translationally, the bacterial form of SRP RNA is missing the elongation arrest domain.
6. Experiments with the protein synthesis inhibitor Puromycin led to the conclusion that initiator methionine charged tRNA can directly enter the P site while no other charged tRNA can.
7. Selectins are proteins found on lymphocytes which allow specific recognition of sugar residues on endothelial mucins. A lectin domain (type of protein domain) would likely be present (is present) on the selectin protein to mediate interaction with a mucin.
8. A GPI anchor (type of posttranslational modification) can serve to anchor a protein to the extracellular face of the plasma membrane.

Part III Matching section (13 points)

The following questions are about carbohydrate 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 for each correct letter, -1/2 point for each incorrect letter: make only good guesses, 13 points maximum, 0 points minimum)

- a. proteoglycan
- b. mucin
- c. N-linked sugar
- d. O-linked sugar
- e. glycosaminoglycan
- f. cytoplasmic GlcNAc

_____ a, b, d, e, f sugar (or has sugars) attached to serine residue of protein

_____ c sugar (or has sugars) attached to asparagine residue of protein

_____ a, e sugar (or has sugars) in (Glu Glu Ser Gly Ser Gly Ser Gly Asp Glu) sequence

_____ c sugar (or has sugars) attached in (Ala Gly Leu Asn Ala Ser Thr Phe) sequence

_____ b, d sugar (or has sugars) attached in (Ser Thr Pro Ser Thr Thr Pro Ser Pro Ser) sequence

_____ c inhibited by tunicamycin

_____ c inhibited by bacitracin

Part IV short answer: use only the space provided, single words or phrases are sufficient (47 points total, points for each question indicated in ())

1.(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, and AP2 binding to Tyr based internalization motif). What is the basis of the discriminating interaction (type of bond)?

OH group (hydroxyl), hydrogen bonding

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

activation of AA , elongation

3.(1) Three features are common to signal sequences directing a protein into the mitochondria and signal sequences directing a protein into the chloroplast. What feature is different and therefore allows differential targeting to these organelles?

Mito signal forms an amphipathic helix, chloroplast signal does not

4.(2) Which two features of a bacterial mRNA specify polypeptide initiation?

shine dalgarno sequence and initiator Met codon (AUG)

5.(2) What are the two sources of genetic code degeneracy?
multiple codons for an amino acid read by multiple tRNAs
one anticodon can read more than one codon (wobble)

6.(2) Name two examples that were covered in class in which a peptide bond is formed between a carboxyl group and an epsilon amino group of lysine residues (isopeptide bond).

bacterial cell wall (peptidoglycan) cross linking
ubiquitination

7.(3) Why are bacterial cells sensitive to glycoprotein synthesis inhibition by bacitracin while eucaryotic cells are not?

bacterial carbohydrate modification occurs extracellularly, eucaryotic carbohydrate modification occurs inside the cell. Bacitracin can not cross membranes

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

Carboxylation and sulfation

9. (4) The GTPase Ran is involved in nucleus-cytoplasm transport. What important feature of the proteins which regulate Ran allows vectorial transport into and out of the nucleus to occur, and what is the activity of these regulatory proteins?

GAP (GTPase activator) localized to (or high concentration in) cytosol
GEF or GNRF (nucleotide exchange factor, nucleotide release factor)) localized to (or high concentration in) nucleus

10.(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

11.(2) What is the activated form of ubiquitin that serves as the ubiquitin donor in ubiquitination?

thioester (-s-) linked to ubiquitin conjugating enzyme

12.(2) What are two potential functions of ATP usage by the 26S proteasome?

unfolding, translocation, substrate binding/recognition

13.(1) Aspartyl proteases have two aspartic acid residues in the active site. HIV protease is an aspartyl protease which contains only a single aspartic acid residue. How can this enzyme function as an aspartyl protease?

works as a homodimer

14.(3) What three features of a cytosolically attached lipid modification affect the affinity of the modified protein for membranes?

length of hydrocarbon chain, degree of saturation, methylation

15.(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**

16.(4) Name two classes of posttranslational modification which can target a protein to the cytoplasmic face of membranes. Name the type of linkage involved (chemical bond between the protein and the modification)?

***Fatty acylation, amide linkage or thioester linkage; or palmitoylation thioester; or myristoylation amide
and
Prenylation or farnesylation or geranylgeranylation, thioether linkage***

17.(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)***

18. You have genetically engineered dynamin (shibire gene product) which lacks its GTPase function but can still bind GTP. You introduce this mutant form of the protein into cells.

a) (1) Where will this protein localize in the cells?

localize to coated pits

b) (3) Why will this protein interfere with normal function even when non-mutant ("wild type") forms of the protein are present?

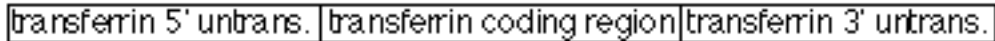
can bind to AP2 but can't perform function so it will compete with normal form and inhibit function

c) (1) What is this inhibitory effect called?

dominant negative

Part V (points as indicated in ()): 18 points total)

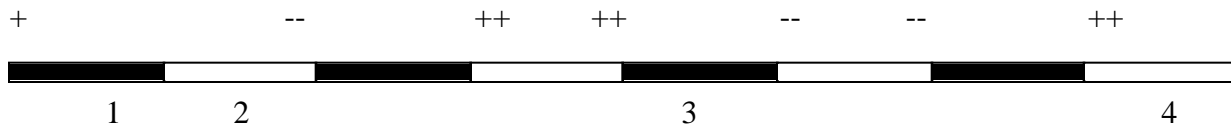
1.(8) Increased iron availability enhances translation of ferritin (iron binding protein), and decreases the translation of transferrin (iron import protein). Aconitase binds the respective mRNAs in the absence of iron, and is released in the presence of iron. Binding, and therefore control of translation, occurs in the untranslated regions. A schematic of the natural mRNAs is shown below.



You engineer the mRNA constructs shown below with a reporter protein coding sequence inserted between the indicated 5' and 3' control regions from transferrin and ferritin mRNA. Will the reporter protein be made in high, medium, or low levels in the absence of iron and in the presence of iron? Place an H, M or L on the lines next to each construct, below the indicated iron status.

	+ iron	- iron			
<table border="1"> <tr> <td>ferritin 5' untrans.</td> <td>reporter</td> <td>transferrin 3' untrans.</td> </tr> </table>	ferritin 5' untrans.	reporter	transferrin 3' untrans.	_____	_____
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transferrin 5' untrans.	reporter	transferrin 3' untrans.			

2.(10) The following hypothetical membrane protein (left to right: amino to carboxyl primary structure) 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 membrane protein into the ER. 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 appropriate attachment sequence is present in all segments)				
GPI transamidase				
central region of translocon				
signal recognition particle				
large subunit of ribosome				
