Name (**Last, First**)_____

Social Security # (last four digits)_____

Graduate _____ or Undergraduate _____

Biochemistry 353

Third hourly Test

April 10, 2000

Part I : _____ / 19

Part II : _____ / 18

Part III: _____ / 10

Part IV: _____ / 53

Total : _____ / 100

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

1. T / F Proteolytic activities within the proteosome are maximal due to maintenance of a low internal pH.

2. 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 serine and threonine residues on proteins.

3. T / F Ran can regulate import and export of proteins from the nucleus because the GTP bound form promotes formation of export complexes, and the dissociation of import complexes, in the nucleus.

4. T / F The lack of a genetic template for glycoprotein synthesis results in heterogeneity of the carbohydrates attached to proteins.

5. T / F The sequence of amino acids which emerge from the ribosome dictate whether a protein will be targeted to the endoplasmic reticulum for insertion by virtue of interaction with signal recognition particle.

6. T / F If two proteins are devoid of cysteine residues a homobifunctional maleimide (SH reactive agent) crosslinker will be an effective crosslinking agent to probe the interactions between these proteins.

7. T / F Diptheria toxin catalytically inactivates eEF-2 via ADP ribosylation of a posttranslationally modified histidine residue present in this eucaryotic elongation factor.

8. 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.

9. T / F Lysosomal function does not require ATP.

10. T / F A major role of Heat Shock Proteins (HSPs) in import of proteins into mitochondria and chloroplasts is the maintenance of proteins in the unfolded state.

11. T / F Coatamer (COP) coated vesicles remain coated from donor to acceptor membrane.

12. 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.

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

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

15. T / F Glucosylation is a sugar modification which can keep a protein from leaving the ER:

16. T / F The adapter protein AP2 links clathrin to transmembrane proteins at the plasma membrane.

17. 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)

18. T / F Ubiquitin is added to other proteins to target them to the lysosome for degradation.

19. T / F Aberrant proteolysis is an underlying factor in Alzheimer's disease.

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. The **hydroxyl** group present on tyrosine is used by several enzymes as a key feature of recognition, allowing discrimination from Phenylalanine.

3. 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.

4. During protein synthesis, mRNA is read in the **5** prime to **3** prime direction.

5. Polypeptides are synthesized from the **amino** to **carboxyl** temini.

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

7. **EF-Tu** serves to protect the amino acyl tRNA linkage, and escorts charged tRNAs to the ribosome.

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

9. Increasing the GTPase activity of EF-Tu will increase the _____**speed / rate**_____ of translation but decreases the _____**fidelity**_____ of translation.

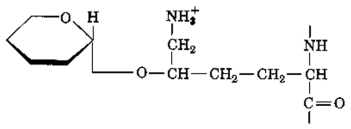
10. Termination of peptide synthesis occurs with the transfer of the peptidyl tRNA in the P site of the ribsome to _____**H_2O**_____.

11. The ribosome has three functionally distinct sites (A, P, and E). Initiator Met tRNA 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.

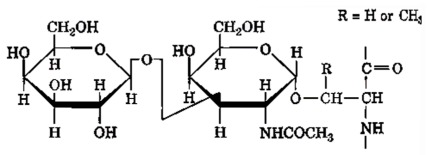
Part III Matching section (10 points)

The sugars below are found in glycoproteins. A through D show the carbohydrate (at left) linked to a protein backbone (at right). E and F show carbohydrates which are linked to other carbohydrates in glycoproteins. Match the letter or letters with the appropriate statements 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, 10 points maximum, 0 points minimum)**

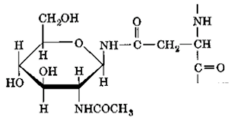
A



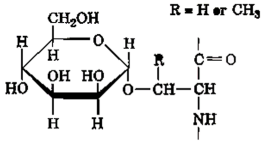
B



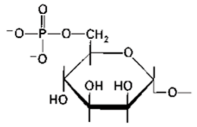
C



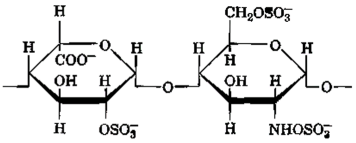
D



E



F



_____B, D, (F)_O-linked sugar

_____C, (E)_ N-linked sugar

_____E_____ present on a protein destined for the lysosome

_____F_____ found on a proteoglycan

_____B_____ contains N-acetylated sugar

_____A_____ found in collagen

_____B_____ β-linked dissacharide

_____F_____ α-linked dissacharide

_____A_____ this carbohydrate linkage forms after posttranslational modification of an amino acid

Part IV short answer: use only the space provided, single words or phrases are sufficient (48 points total, points for each question indicated in ())

1. (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

2. (6 points) List the three types of RNA involved in protein synthesis and their function

	RNA	**function**
1.	**messenger**	**informational / template**
2.	**transfer**	**adapter / activated intermediate**
3.	**ribosomal**	**structural / catalytic**

3. (8 points) How many signals are present in a protein destined to receive a GPI anchor? Where are these signals located and what are the general features, functions, and fates of these signals?

Two signals: The first is at the amino terminus which directs the protein to the ER for cotranslational insertion (+ charge followed by hydrophobic sequence followed by small residue at cleavage site), this signal is removed. The second is a hydrophobic stretch at the carboxyl terminus which serves as a temporary membrane anchor, it is also removed.

4. (8 points total as indicated) Interactions between SNARE proteins on donor and acceptor membranes are responsible for intracellular membrane fusion events.

a) (2 points) 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)?

complexes occur in "trans" prior to fusion (before), and in "cis" following fusion (after)

b) (2 points) What protein is involved in the formation of "trans" SNARE complexes, and what protein is involved in resolving (dissociating) "cis" SNARE complexes?

Rab GTPases play a role in forming trans SNARE complexes while NSF plays a role in resolving cis SNARE complexes to regenerate separate v- and t- SNARES for subsequent fusion events.

c) (4 points) Briefly, what are the two major differences between the mode of viral fusion protein action in membrane fusion and the role of SNARE proteins in membrane fusion (think where are the proteins located and how are they activated)

The viral fusion protein is donated from a single bilayer (the viral envelope) and is activated by a change in pH, while the SNARE proteins are located on the two, initially separate, membrane compartments to be fused and their activity is regulated by other proteins.

5. (4 points) Endocytosis of iron bound transferrin involves two pH dependent steps which allow internalization of iron but recycling of transferrin and the transferrin receptor. Where do the two pH dependent steps occur, and what is the outcome of the pH change (relative to iron and transferrin)?

endosome acidifies which causes release of iron from transferrin
fusion of apotransferrin-tranferrin recpetor containing vesicle with plasma membrane increses pH causing release of transferrin from transferrin receptor

6.(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).

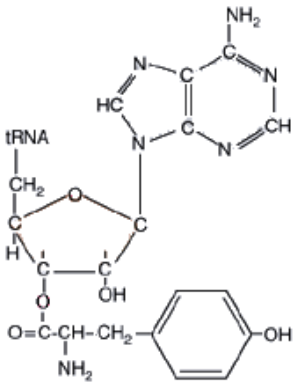
covalent modification of active site residue
nonhydrolyzable (slowly hydrolyzable) substrate analog

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

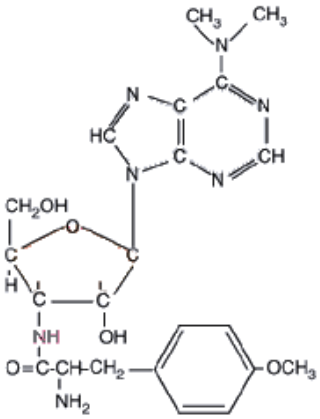
protonation of carboxylates
intramolecular salt bridges lost

8. (4 points total as indicated) Mark the features on the structures as indicated below.

Tyrosyl tRNA



Puromycin



a) (2 points) Assuming that the molecules depicted above are present in the A site of the ribosome (obviously not at the same time!), draw an arrow pointing to the functional group which will attack the peptidyl tRNA present in the P site of the ribosome (one arrow for each molecule).

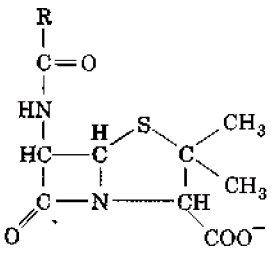
b) (2 points) Assuming that the depicted tyrosyl tRNA is present in the P site of the ribosome (the other amino acids of the peptidyl tRNA are not shown), draw a circle around the functional group which would be attacked by the amino acid present in the A site during the peptidyl transferase reaction. Also, draw a circle around the functional group of puromycin which disallows this peptidyl transferase reaction.

9. (3 points as indicated) The structure of penicillin is shown below at right.

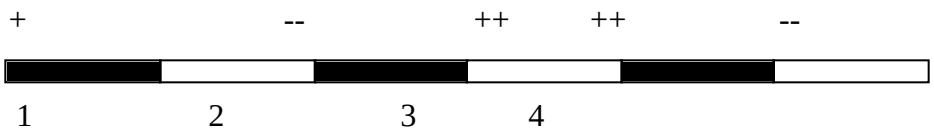
a) (2 points) What reaction in bacterial cell wall biosynthesis is inhibited by this compound?

cross linking of peptidoglycan chains

b) (1 point) Draw an arrow pointing to the bond which is subject to hydrolysis by β-lactamase.



10. (6 points) 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)				
central region of translocon				
signal recognition particle				