

Name \_\_\_\_\_

Name (Last, First) \_\_\_\_\_ **KEY** \_\_\_\_\_

Graduate \_\_\_\_\_ or Undergraduate \_\_\_\_\_

**Biochemistry 353, Spring 2002 Final Exam, May 4, 2002**

Before you start, PRINT your name and social security number on the top of this page. Be sure to print your name on the top of each page. Notes of any kind are **NOT** permitted.

Confine your answers to the space provided. Only answers in the space provided will be graded. If you cross out an entire answer, you may write your answer in the SAME amount of space on the back of the page. However, you **MUST** indicate on the **FRONT PAGE** of the exam which questions have been answered on the back of a page or they will **NOT** be graded.

**The exam has 14 numbered pages. Check to make sure that you have all of the pages.**

Part I : \_\_\_\_\_ / 24

Part II : \_\_\_\_\_ / 24

Part III: \_\_\_\_\_ / 20

Part IV: \_\_\_\_\_ / 10

Part V : \_\_\_\_\_ / 24

Part VI: \_\_\_\_\_ / 19

Part VII: \_\_\_\_\_ / 79

Total : \_\_\_\_\_ / 200

**Part IV True / false section: circle appropriate letter (10 points; 1 point each)**

1. T / F Nuclear import occurs by a process of vesicular transport.
2. T / F SNARE proteins involved in membrane fusion events are the targets of cholera toxin produced by bacteria.
3. T / F Isopeptide bonds are found in bacterial cell walls and in ubiquitinated proteins.
4. T / F All of the carbon atoms in cholesterol are derived from acetate.
5. T / F G-protein coupled receptors are guanine nucleotide exchange factors for heterotrimeric G proteins.
6. T / F Inactivating a positive regulator of the cell cycle results in abnormally large cells.
7. T / F The translocon creates a pore through the endoplasmic reticulum (ER) membrane which allows N-linked core carbohydrates to flip across the membrane from their site of synthesis in the cytoplasm to the site of attachment to proteins in the lumen of the ER.
8. T / F Signal recognition particle (SRP) binding to the signal sequence of a secreted protein arrests translation and targets the protein to the plasma membrane for secretion.
9. T / F Because the primary amino acid sequence of a membrane protein determines its topology in the membrane, detergent solubilized membrane proteins incorporated into lipid bilayers in vitro adopt their correct orientation.
10. T / F Acetate and Choline are the substrates used but choline-acetyl-transferase to synthesize acetylcholine.

**Part V Fill in the blank section (24 points; 1 point for each blank)**

1. **cyclin (mitotic cyclin better)** and **cyclin dependent kinase (cdc2 or cdk or p34 OK)** are the two protein components of Maturation Promoting Factor (MPF; also called Mitosis Promoting Factor). **Thr kinase** \_\_\_\_\_, and **Tyr Kinase** \_\_\_\_\_, and **Tyrosine phosphatase** \_\_\_\_\_ are the three enzymatic activities needed for activation of MPF, while termination of MPF function involves **cyclin destruction by ubiquitin/proteasome pathway** \_\_\_\_\_.
2. **puromycin** \_\_\_\_\_ is the protein synthesis inhibitor which was instrumental in developing the concept of unique sites in the ribosome. In the absence of translocation this inhibitor can be transferred to **initiator Met** \_\_\_\_\_ but not **growing polypeptide chains** \_\_\_\_\_.
3. Mutating a **Y** (amino acid residue; single letter code) to a **F** (amino acid residue; single letter code) would be the best way to test the role of a tyrosine as a potentially important target for phosphorylation.
4. **EF-Ts** \_\_\_\_\_ is the GEF (guanine nucleotide exchange factor) for EF-Tu.
5. The active site residue of caspases is **cysteine** \_\_\_\_\_ and these enzymes cleave after **D (aspartate)** \_\_\_\_\_ residues.
6. Small G-proteins are notoriously poor enzymes. They possess a weak **GTPase** \_\_\_\_\_ activity (enzymatic activity). Enzymatic activity occurs at an appreciable rate due to the activity of **GTPase activating proteins (GAPs)** \_\_\_\_\_.
7. **GAG** \_\_\_\_\_ and **POL** \_\_\_\_\_ and **ENV** \_\_\_\_\_ are the three genes common to all retroviruses.
8. **FAS** \_\_\_\_\_ or **GranzymeB** \_\_\_\_\_ plus **perforin** \_\_\_\_\_ are the two mechanisms used by a T-Cell to initiate apoptosis in a target cell.
9. **cytochrome C** \_\_\_\_\_ and **ATP** \_\_\_\_\_ are the cofactors required to generate an active complex containing Apaf which stimulates apoptosis.

**Part VI short answer: use only the space provided, single words or phrases are sufficient (19 points total, points for each question indicated in ( ))**

1.(3) What are two steps in protein synthesis that sometimes result in GTP hydrolysis which is non-stoichiometric with polypeptide chain elongation, and what is gained by this extra expenditure of energy?

**tRNA charging (hydrolytic site activity in AA-tRNA synthetase)  
elongation (delivery of aminoacyl-tRNA into A site if wrong AA is delivered)  
increased fidelity.**

2.(2) What are the two sources of degeneracy in the genetic code?

**wobble base pairing  
multiple codons (tRNAs) for some amino acids**

3.(3) Signal transduction is often characterized by cascades of enzymes (i.e. KinaseKinaseKinase->KinaseKinase->Kinase or ProteaseProteaseProtease->ProteaseProtease->Protease). What are three advantages (properties) of these kinds of systems?

**amplification  
more opportunity for regulation  
more sites for integration of pathways**

4.(5) A major contributor to the transmembrane potential across the plasma membrane of cells (including neurons) is an electrogenic, ion pumping, ATPase. What ions are pumped, which direction are they pumped and what makes this electrogenic?

**Na<sup>+</sup> out, K<sup>+</sup> in; more Na<sup>+</sup> than K<sup>+</sup> makes a charge differential**

5.(2) Acetylcholine can alter cAMP levels in some neurons but in other neurons it causes changes in plasma membrane ionic permeability. How can a single neurotransmitter do different things to different cells?

**the cells have different receptors; one is a 7TM protein which interacts with a heterotrimeric G protein, the other is pentameric ligand gated ion channel.**

6.(4) Binding of the G alpha subunit of a heterotrimeric G protein to GTP is partially mediated by switch regions I, II, III of the G alpha protein. What protein-protein interactions are also mediated (at least in part) by these regions of the G alpha subunit, and in which nucleotide bound states do these interactions occur?

**binding to the beta-gamma subunit occurs when GDP is bound  
binding to effector proteins (e.g. adenylate cyclase or phospholipase C) occurs when GTP is bound**

**Part VII essay: use only the space provided. (79 points total, points for each question indicated in ()); points may be deducted for incorrect statements; please take your time and think before you write). As always brevity is a virtue, many of the questions can be answered with key terms and phrases.**

1.(6) How do changes in pH facilitate the normal cycling of transferrin through the endocytic pathway (where do these changes occur, and what is the outcome 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.(5) Snare proteins mediate a critical aspect of intracellular membrane fusion events in eucaryotic cells and are thought to function analogously to the HA fusion protein which mediates fusion of viral membrane with host cell membrane. What are the major differences in the organization, and means of control, of these fusogenic molecules/complexes?

**HA is a single protein present in the viral membrane. It is maintained in an inactive conformation. Changes in pH alter the conformation allowing the protein to incorporate into the host cell membrane (the HA protein now spans the two bilayers to be fused) and drives the fusion event. (cis-> trans-> cis)**

**Snare complexes are formed by multiple proteins at least one of which is in the donor membrane the other of which is in the acceptor membrane. Protein-protein interactions regulate the conformational changes in this complex which drive membrane fusion. (trans-> cis)**

3.(5) Diisopropylphosphofluoridate (DIPF) inactivates serine proteases, and its analogue Sarin (a nerve gas) inactivates the catalytically similar enzyme Acetylcholinesterase (AChE). Bovine pancreatic trypsin inhibitor (BPTI) and  $\alpha$ 1-antitrypsin are also effective in inhibiting serine proteases (trypsin and elastase respectively), but do so by a mechanism which is from the mechanism employed by DIPF/sarin. Please describe the general mechanism of action of these two classes of inhibitors.

**DIPF and sarin form a covalent linkage with the active site serine residue irreversibly inactivating the enzyme, while BPTI and antitrypsin are peptides which mimic a substrate and bind in the active site but are not (or extremely slowly) cleaved by the enzyme.**

4. (6) Describe the linkages (protein-lipid linkage chemistry and location in protein) used in generating fatty acylated (two types) and prenylated (one type) proteins.

**Fatty acylation involves either a thioester linkage to cysteine residues found anywhere in a protein or an amide linkage to an amino terminal glycine residue while prenylation involves a thioether linkage to a carboxy terminal carboxy-methylated cysteine residue.**

5.(6) Compare and contrast the carbohydrate present on a proteoglycan with those present on a protein with N-linked carbohydrate. Please limit your answer to the defining characteristic of the sugars (general organization and important modifications, not the specific sugars), and the sugar-protein linkage.

**Proteoglycan: linear, repeating disaccharides, highly negatively charged (sulfated and carboxylated), amino sugar containing, link tetra saccharide attached to S residues (ether linkage) present in SG repeat sequence flanked by acidic residues**

**N-linked: branched, GlcNAC (or NAG or N-acetylglucosamine) attached to N in NxS/T sequence (amide linkage)**

6.(8) Signal transducing receptor systems can consist of two different receptor molecules, one of which is ligand binding the other of which generates the signal. One signal enhancing property that derives from this arrangement is due to a reduction in the degrees of freedom experienced by the ligand when it is bound to the ligand binding receptor for subsequent presentation to the signaling receptor. What is meant by reduced degrees of freedom and explain the relevant reductions.

**Degrees of freedom refers to a molecule's rotational and translational (movement through space) mobility**

**Translational degrees of freedom are reduced from three dimensions to two dimensions (tethered to the membrane can only diffuse in x and y but not z dimension)**

**Rotational degrees of freedom are reduced from three to one (ligand receptor complex can only spin on the axis normal to the membrane)**

7.(6) The ribosome, the nucleosome and viruses are three nucleoprotein particles that were discussed in class. Briefly describe the relationship between the nucleic acid and the protein components (general organization) of these three nucleoprotein particles.

**Nucleosome is protein core surrounded by nucleic acid**

**Virus is nucleic acid (and protein) core surrounded by protein**

**Ribosome has protein interdigitated with the nucleic acids like bricks and mortar**

8.(7) Describe the features (types of amino acids, general secondary structure if relevant, and location in the protein) of the signals which direct proteins to the 1) mitochondria, 2) lysosome, and 3) nucleus.

**mitochondria signal is present at the amino terminus and consists of basic and hydroxylated AAs organized as an amphipathic helix. Lysosome signal is a Mannose-6-phosphate tag present on carbohydrate (sugar; glycosylation) residues added to the core protein. Nuclear localization signal is stretch of basic residues which can be anywhere in the primary sequence of the protein**

9.(8) SREBP is a membrane bound transcription factor which is sensitive to cholesterol concentration. Describe the major steps used in regulating this transcription factor in response to cholesterol levels.

**In the absence of cholesterol SCAP and SREBP transit to the golgi where SREBP is cleaved by site 1 protease followed by site 2 protease cleavage which frees the transcription factor to enter the nucleus. Cholesterol binds SCAP which inhibits SCAP's ability to transport SREBP from the ER to the golgi.**

10. What effect upon PKA activity would you expect if you  
a) (2) mutated the pseudosubstrate sequence of the PKA regulatory subunit so that it could not bind the catalytic subunit of PKA?

**leave catalytic subunits free and catalytically active**

b) (2) mutated the cAMP binding sites of the PKA regulatory subunit so that they could not bind cAMP?

**make cAMP unable to relieve inhibition, PKA would be catalytically dead**

c) (2) Which, if either, of the above mutants would act as a dominant negative if overexpressed in the presence of the wild type versions of PKA subunits (assume that it is NOT possible to express the mutants at levels which can interfere with cytosolic cAMP concentrations)?

**cAMP bind site mutant is dominant negative, pseudo substrate mutant is not**

11.(8) You have a small molecule ligand which activates a Heterotrimeric G-protein containing G $\alpha$ . List or draw a schematic of the flow of protein-protein interactions from ligand receptor binding to protein kinase A (PKA or A kinase) activation.

**ligand + receptor changes receptor  
ligand receptor complex catalyzes binding of GTP to G $\alpha$  and release of GDP  
G $\alpha$ -GTP releases G $\beta$ - $\gamma$   
free G $\alpha$ -GTP stimulates adenylyl cyclase->cAMP is produced  
cAMP binds regulatory subunits of PKA  
regulatory subunits dissociate from PKA catalytic subunit  
catalytic subunit is now free active kinase**

12.(8) Receptor tyrosine kinase activation can result in phosphorylation of transcription factors and changes in gene expression. Cytokine receptor activation can also alter gene expression. In general terms, compare and contrast the signaling induced by these two classes of receptors, and identify which of these mechanisms is similar to the mechanism employed by TGF beta type ligands which signal through Ser/Thr transmembrane kinases.

**RTKs activate a complex cascade involving ras activation and a cascade of kinases which ultimately phosphorylate transcription factors. Phosphorylation of the transcription factors is far removed from the initial activated receptor. Cytokine receptor activation involves the activation of JAKs (janus kinases) which in turn recruit and phosphorylate STATs which are then themselves activated transcription factors. Phosphorylation of the transcription factors occurs at the activated receptor. S/T kinase activation by TGF beta type ligands is similar to cytokine activation (relatively direct activation of transcription factors : smads)**