Chemistry 532: Exam 1
Spring 2001 (100 points)

Name Key

Answer the following questions with very brief statements; correct responses to many of the questions need only one or two short sentences.

1. (10 points) Describe the general function and structural features of G-protein-coupled receptors (GPCRs).

They are extracellular receptors that, when bound by ligand, cause an intracellular G-protein to dissociate from the receptor, bind GTP and then activate other proteins such as cAMP Cyclase or ion channels. It is an integral membrane protein that consists of seven transmembrane helices in an approximatey circular array.

2. (10 points) What chemical transformations do retinoids undergo in the eye?

- cis to trans isomerization across the double bond
- reduction-oxidation of aldehyde-alcohol
- esterification of the alcohol

3. (10 points) In a recent report in *Science* the authors used a FRET assay to monitor the activation of a yeast GPCR by cAMP, which is a chemoattractant for yeast. Yeast lacking two of the three G-protein subunits were transfected with the missing subunits. One was fused to a fluorescent protein that is excited at 440 nm and it emits at 490 nm. The second subunit was fused to a fluorescent protein that is excited at 490 nm and it emits at 540 nm. The fluorescence at 540 nm (excitation at 440 nm) was recorded for single cells. Upon addition of cAMP, the fluorescence intensity decreased reaching a steady state in about 30 s. Upon removal of cAMP the fluorescence increased back to the original value in 30 s. Describe what is happening inside the yeast cells that accounts for the observed changes in fluorescence.

The fluorescence at 540 nm is observed because the two subunits are bound to the GPCR giving energy transfer. Upon ligand binding - the G-proteins dissociate and are no longer close enough to do FRET. Upon removal of ligand - the re-bind.

4. (10 points) Describe the major classes of constituents in growth media for mammalian cells and their general purpose.

- salts — maintain osmotic balance ie size, volume
- nutrients — carbon sources used for energy production ie glucose
- vitamins — precursors for biosynthetic reactions
- serum — growth hormones to promote mitosis

5. (10 points) Describe how flow cytometry assays can be used to monitor cell cycle.

Cells are lysed and nuclei are stained with a fluorescent intercalator such as PI. The mean fluorescent intensity measured is proportional to the amount of DNA, which is a function of cell cycle.

6. (10 points) A Chinese hamster (CHO) cell line was transfected with a plasmid that encodes for a GPCR and an appropriate luciferase reporter gene. Flow cytometry of the transfected cell line using an antibody specific to the GPCR confirms that it is expressed at high levels. Control experiments confirm that the target promoter and luciferase gene are functional. However, treatment of the cells with a known ligand for the GPCR does not produce a luciferase response. Suggest a reason for the lack of a luciferase response.

The CHO cell line is lacking one of the proteins required for signaling ie the needed G-protein

7. (10 points) A statement made in class is not entirely true- there is an excellent example of a cell membrane receptor whose signaling appears to be dependent on a conformational change. If certain cells are super-transfected with the m1-muscarinic receptor, the extremely high level of expression leads to a low level of constitutive signaling by the receptor. If an agonist for the receptor is then added, the level of signaling increases, as expected. The separate addition of most antagonists to the cells has no effect on signaling (also expected since there is no agonist to compete with). However, the addition of some known antagonists blocks the low level of constitutive signaling even though no agonist is present. Describe how this observation supports the hypothesis that the m1-muscarinic receptor undergoes a conformational change to trigger signaling when bound by an agonist.

$R_i \overset{+L}{\rightleftharpoons} R_a$ The receptor alternates between two conformations, one is active to signaling. The agonist ligand stabilizes the active form. At high levels of expression, enough are in the active form to signal. The "weird" antagonist ligands stabilize the inactive form and, thus, shift the equilibrium to R_i.

8. (10 points) One of the reasons why kinase enzymes may have been selected for use in signal transduction pathways is that kinase-mediated phosphorylation cascades can be easily regulated. Describe how a phosphorylation cascade can be regulated and why signal transduction pathways mediated by proteases are not so easily regulated.

The cell has plenty of ATP which can be used to phosphorylate -OH groups. The cell uses phosphatases to reverse the reaction (hydrolysis) and, thus, regulate the cascade. When a protease hydrolyzes an amide bond, there is no easy way to reverse the reaction and, thus, it is difficult to regulate.

9. (10 points) Name the two major subsets of T cells and their general role in the generation of an immune response.

CTLs - cytotoxic T cells directly kill virus infected cells and tumor cells

T_h Cells - helper T cells secrete cytokines required for the function of CTL's and B cells

10. (10 points) What is the role of the MHC I protein in an immune response to viruses.

MHC I presents peptides derived from proteins inside the cell. A cell infected with a virus will express MHC I with viral peptides - these will trigger CTLs to kill the cell.