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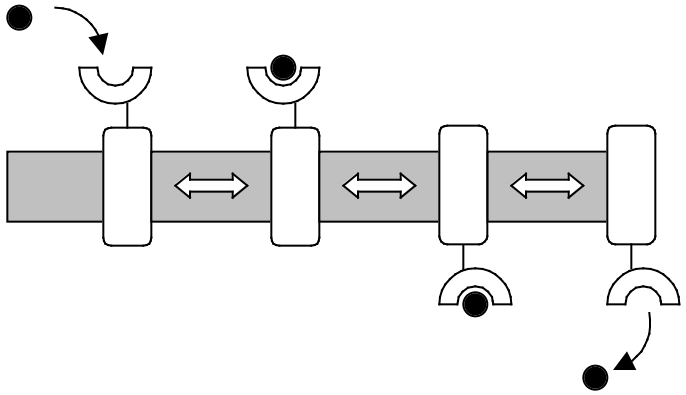
7.013 Problem Set #7 4/28/00

Answers to this problem set are to be turned in at the box outside 68-120 by 12:30 PM (by the clock in 68-120) on 5/5/2000. Problem sets will not be accepted late. Solutions to this problem set will be posted on the web by late 5/5/2000. (http://web.mit.edu/7.01x/www/).

Question 1

GLUT1 is a plasma membrane bound transporter of glucose in mammalian cells. It has two conformations, one with its glucose binding site pointing into the exterior of the cell, and the other with its glucose binding site pointing into the cytoplasm:



Like any enzyme, each of the reaction above is reversible, as indicated by the double arrow.

a) Initial experiment indicate that the K_m for the enzyme for glucose is 1.5 mM. Blood glucose level is normally 5 mM. Will glucose bind to the receptor? Why?

b) The reversibility of the glucose transporter means that it can transport glucose out of the cell into the bloodstream. Liver cells can make glucose. Under what condition will it be favorable for glucose to be transported into the bloodstream?

c) In an experiment in which there are a lot of glucose molecules inside the cell, adding radioactive glucose to the "bloodstream" reveals really rapid uptake of the radioactive glucose into the cells (more rapid than without glucose already inside the cell).

Explain this phenomenon.

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Question 2

You are studying a patient with familial hypercholesterolemia (FH). The patient (FH1) is a 29 year old woman with homozygous FH, and the first human recipient for FH gene therapy. In particular, this woman suffered a massive heart attack with permanent damage at the age of 16 revealing the seriousness of this heritable disease. In the following problem, you are conducting a new and controversial experiment called "Gene Therapy" in an effort to find a clinical approach to reduce the seriousness of this disease.

PROCEDURE:

1) 15% of patient FH1's liver was removed and the liver tissue sample was then dissociated into individual hepatocytes (liver cells) and cultured in petri dishes.
2) The cultured cells were transduced (infected) with a retrovirus carrying the DNA for the gene which you think might solve FH1's problem. (Note: this virus was modified so that is will infect cells without killing them. It cannot become lytic.)
3) After two days in culture, the infected hepatocytes were placed back into the patient's liver.

RESULTS:

1) 20% of the infected hepatocytes express the introduced gene.
2) A sample of FH1's liver, removed 18 months after the procedure, contained cells that expressed the introduced gene in the ratio of: (1 expression cell) / (approx. 1,000 – 10,000 total hepatocytes).
3) The following LDL concentrations were determined in FH1's bloodstream:*

Pre-Gene Therapy Without Lovastatin**	Post-Gene Therapy Without Lovastatin	Post-Gene Therapy With Lovastatin
482 mg / dl	404 mg / dl	356 mg / dl

· * The average, "normal," LDL level in an adult is 125 milligrams per deciliter (mg / dl).
· ** Lovastatin is an enzyme inhibitor of HMG CoA reductase, a key enzyme in cholesterol synthesis.

a) What gene is being introduced into the hepatocyte of FH1?

b) How can viral infection maintain expression of the introduced gene after the hepatocytes are returned to the liver? Why is the introduced gene still expressed 18 months after viral infection?

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Question 2 continued

Patient FH1 was unresponsive to conventional drug therapy for heterozygous FH patients (such as taking Lovastatin, and enzyme inhibitor of HMG CoA reductase, or taking bile acid binding resins) to reduce LDL in the blood before gene therapy:

c) Why was FH1 unresponsive to conventional therapy before the "Gene Therapy" experiment?

d) Why is FH1 responsive to conventional therapy after the "Gene Therapy" experiment?

e) The promoter driving the transcription of the introduced gene is not the normal promoter. Why might this seem to contradict what is given is the section above? Explain (using a little imagination) how else, besides through transcription, the gene expression of the introduced gene might be regulated?

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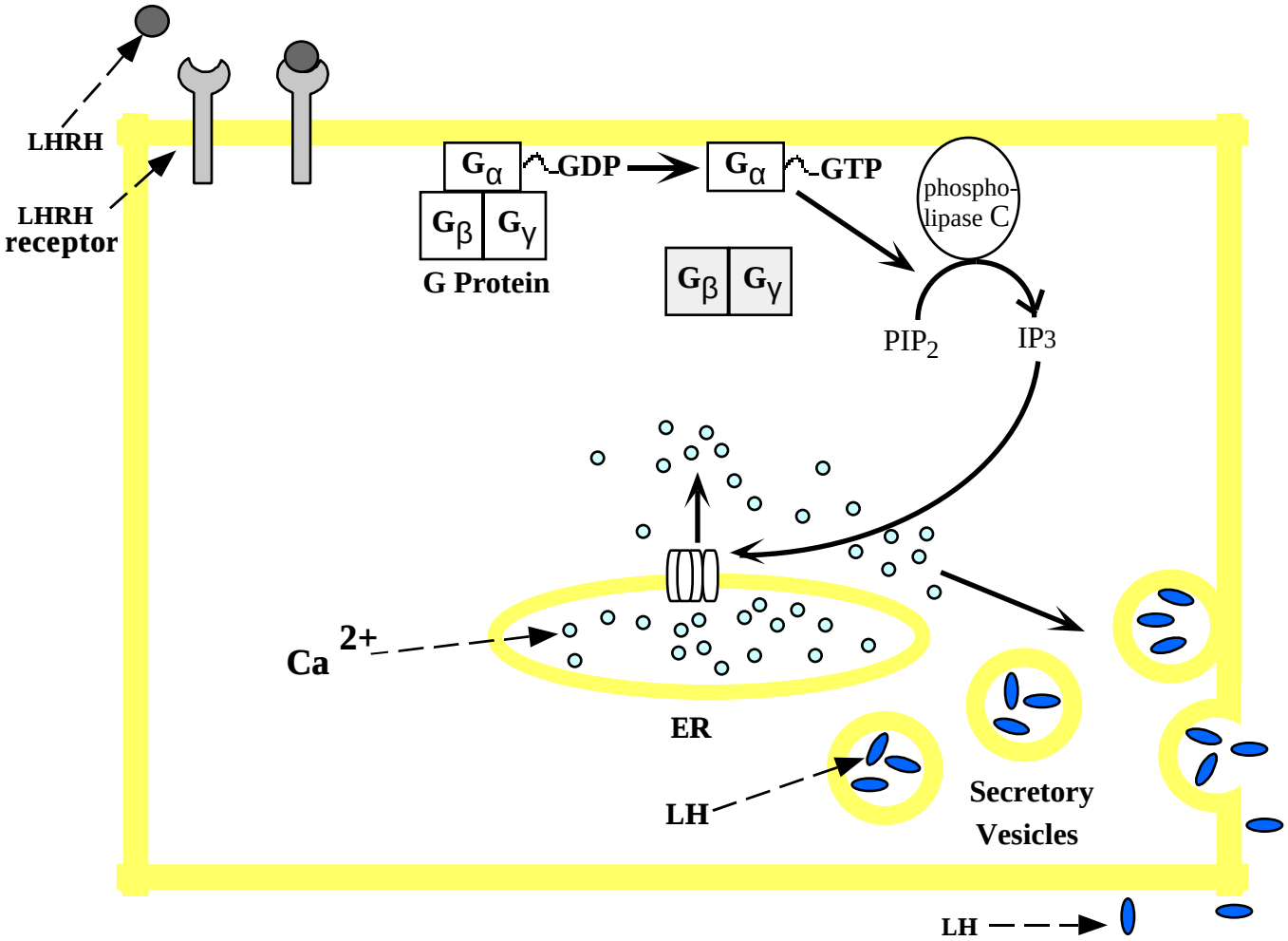
Question 3

Shown below is the signaling pathway for the secretion of Luteinizing hormone (LH) in pituitary gland cells in response to Luteinizing Hormone Release Hormone (LHRH).



1) LHRH binds and activates the LHRH receptor.

2) The active LHRH receptor activates a G-protein that results in the exchange of GDP to GTP on the $G\alpha$ subunit and the dissociation of the $G\alpha$ subunit from the $G\beta G\gamma$ subunits. Hydrolysis of GTP inactivates $G\alpha$ and leads to the reassociation of the complex.

3) The $G\alpha$-GTP subunit activates phospholipase C, which catalyzes the conversion of PIP_2 to IP_3.

4) IPT promotes release of calcium (Ca^{2+}) from the endoplasmic reticulum, a site of internal stores of calcium in the cell.

5) A rise in cytoplasmic Ca^{2+} leads to the fusion of secretory vesicles containing LH with the plasma membrane and release of LH.

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Question 3 continued

a.) In this system, Ca^{2+} is acting as a "second messenger." What is another example of a second messenger that we have discussed in class?

b.) You isolate 3 mutant cell lines, each of which alters the secretion of LH. Predict the LH secretion phenotype for each of the mutants (choices include continuous, higher, lower, or no secretion).

i.) mutant 1 = phospholipase C cannot convert PIP_2 to IP_3

ii.) mutant 2 = the mutant LHRH receptor binds to LHRH less strongly than the wild-type LHRH receptor

iii.) mutant 3 = the G_α subunit of the G protein hydrolyzes GTP at half the rate of the wild-type subunit

c.) Give 2 examples of mutations which will result in continuous secretion of LH.

d.) Adrenaline is another hormone which acts through a receptor / G-protein / second messenger system to cause changes in cells. However, adrenaline has no effect on the secretion of LH in the anterior pituitary cells. Explain this observation.

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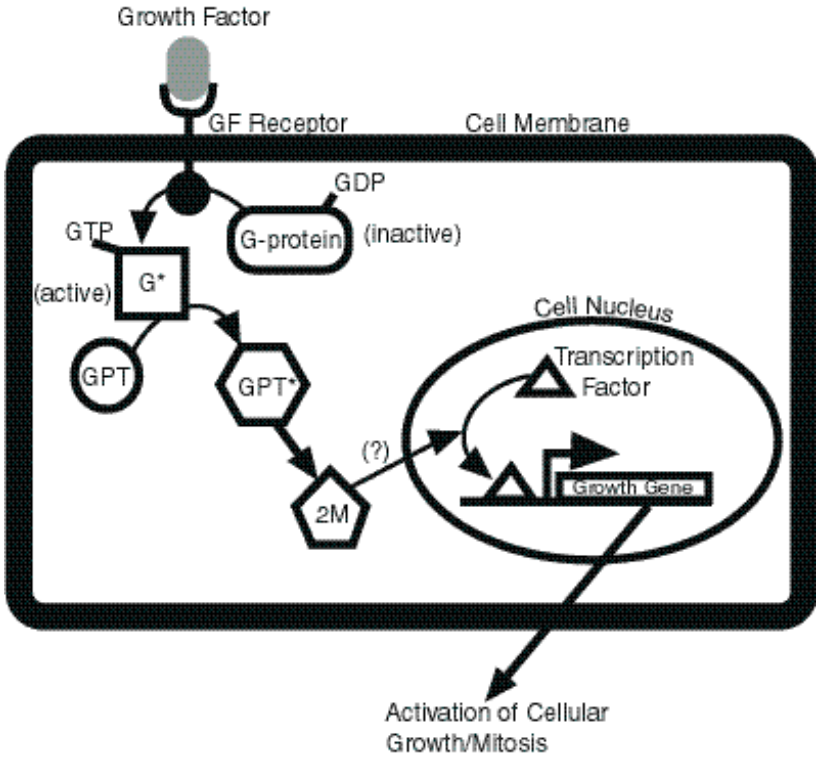
Question 4

The growth of specific types of cells in the human body is regulated by cell-type-specific growth factors (e.g., in a wound, surrounding skin cells respond to secreted growth factors by proliferating to repair damage). These growth factors are often small peptides secreted by one cell type that trigger another cell type to proliferate via a G-protein cascade. Shown below is a diagram of a typical pathway for control of cell growth by extracellular growth factors.



The process of activation of cell growth is described as follows:

1) The growth factor is secreted by a secreting cell and diffuses to the receptor on the target cell.

2) Binding of the growth factor to the receptor activates the receptor.

3) The activated receptor activates a G-protein (G → G*). The G protein binds a GTP molecule in its activated state.

4) The activated G-protein (G*) directly causes the activation of a G-Protein Target (GPT). The two most common types of G-Protein targets are:

    a. Protein kinases: These activate other proteins by phosphorylating them.

    b. Enzymes that produce second messengers, like adenylate cyclase.

    The net result of either of these is the production of a second messenger molecule (2M), either a phosphorylated protein, or a small molecule like cAMP.

5) The second messenger activates transcription factors in the nucleus through mechanisms that are not well understood. Sometimes, existing transcription factors are activated and sometimes, new transcription factors are expressed.

6) The activated transcription factors cause the transcription of the genes that are involved in controlling cell growth and division.

7) The expression of these genes leads to the production of proteins that cause cells to grow and divide.

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Question 4 continued

Cancer is often the result of uncontrolled cell division – that is, cell division even in the absence of growth factors. Mutations that alter the cellular components that control cell growth will often lead to cell growth in the absence of growth factors. Mutant alleles of these genes are called "oncogenes." A tumor is a clone of cells growing uncontrollably. It consists of the descendants of a single mutant cell that has lost control of growth because of a mutation in a gene encoding one of the proteins described in the pathway above.

1) For each of the following components (a through d) explain:
   - How could the component be altered to cause the cell to grow in the absence of growth factor and lead to cancer? What region (regulatory or coding) of the gene encoding the component would be altered by such a mutation?
   - Would the cancerous phenotype of the mutant gene be dominant or recessive to the non-cancerous phenotype of the wild-type gene?

   a) Growth factor receptor:

   b) G-protein:

   c) G-protein target:

   d) Growth factor: