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**MCB130 midterm exam**

1) The exam has 6 pages (not including this page). Check that you have all 6 pages

2) Please write your name and SID on the top of each page. The pages will be separated for grading

3) Restrict your answer to the space provided. If you run out of space for some reason, continue on the back of the same page, NOT on the next page. The pages may be separated for grading

4) Please write clearly and legibly. The grader has to be able to read your answer without your assistance!

Good Luck!

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|---|---|
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| **TOTAL** | **/90** |

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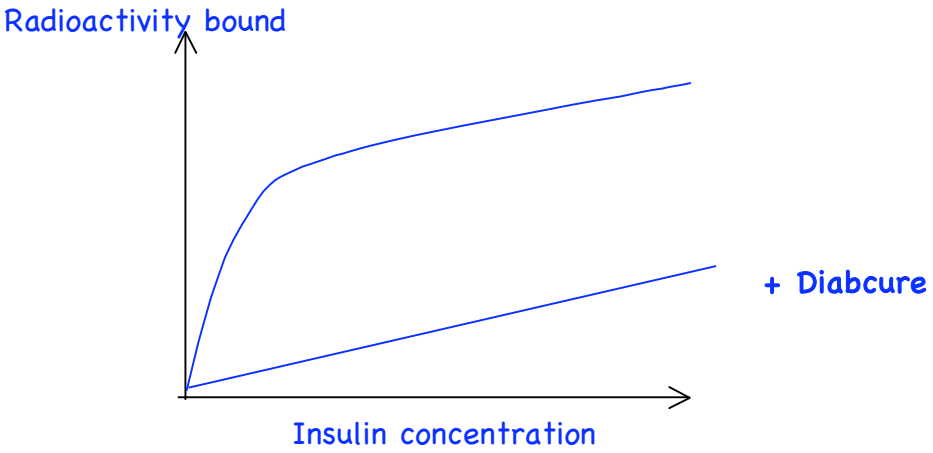
1(a) (6 points) You add radioactively-labeled insulin to liver cells that have 30,000 insulin receptors per cell. The $K_d$ of the interaction between insulin and its receptor is $10^{-8}$M. On the axes provided, <u>draw a single curve</u> that shows how the binding of radioactive insulin to the cells would change (as directly measured in your experiment) with increasing concentrations of radioactive insulin. Remember to label the axes.




1(b)(4 points) A pharmaceutical company has developed a new drug Diabcure that competes with insulin for binding to its receptor. The $K_d$ for the interaction between Diabcure and the insulin receptor is $10^{-11}$M. You repeat the binding experiment but this time you always mix the radioactive insulin with an equal concentration of Diabcure. Draw on the same axes, the expected binding of radioactive insulin in the presence of Diabcure (label this '+ Diabcure') .

2 (6 points) Are the following statements about the insulin receptor True or False

(a) (2 points) The insulin receptor has 7 transmembrane domains

False

(b) (2 points) The insulin receptor dimerizes upon ligand binding

False

(c) (2 points) The insulin receptor has enzymatic activity

True

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3)(6 points) A newly developed drug called Niagra enters cells and blocks the interaction of $Ca^{2+}$ with calmodulin. What would be the effect of Niagra on the following (increase, decrease or unchanged)?

a) (2 points) Activation of the $Ca^{2+}$/calmodulin-dependent protein kinase (CaM-kinase)
Decrease

b) (2 points) The generation of diacylglycerol (DAG)
Unchanged

c) (2 points) The activity of the pump that pumps $Ca^{2+}$ out of cells
Decreased

4) (9 points) In an experiment with purified proteins, radioactively labeled GTP is bound to Ras. RasGAP is added to the reaction. After a period of incubation you measure the radioactivity that remains bound to Ras. Would it increase, decrease or be unchanged if:

a) (3 points) The γ-phosphate group of GTP is radioactively labeled

Decrease

b) (3 points) The α-phosphate group of GTP is radioactively labeled

Unchanged

c) (3 points) Both the α– and γ– phosphate groups of GTP are radioactively labeled

Decrease

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5) (6 points) When you add the drug rapamycin to cells, what would happen to the following? Answer only whether they would increase, decrease, or stay unchanged.

a) (2 points) Translation of mRNA

Decrease

b) (2 points) Phosphorylation of Akt(PKB) by Tor

Unchanged

c) (2 points) The percentage of Rheb that is GTP-bound

Unchanged

6) (8 points) Explain how dimerization of the EGF receptor results in the recruitment of Grb2.

Dimerization of the receptor brings the two kinase domains close to each other.

One kinase domain activates the other by an allosteric mechanism (dont need to use the word allosteric).

The activated kinase domain phosphorylates tyrosine residues on the C-terminal tail of the other EGFR molecule in the dimer (intermolecular transphosphorylation).

Grb2 binds to a phosphotyrosine residue on the EGF receptor via its SH2 domain.

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7) (9 points) Regarding MAP kinase (write one sentence for each answer)

a) (3 points) How does MAP kinase dimerization affect its localization?

It allows it to enter the nucleus

b) (3 points) How does MEK activate MAP kinase?

By phosphorylating it on threonine and tyrosine residues in its activation loop. (one point for each underlined word/phrase)(T-loop is OK instead of activation loop)

c) (3 points) Why is there very little amplification in the MAP-kinase activation pathway that responds to mating pheromone in yeast?

The three kinases in the cascade are held together by a scaffold.

8) (10 points) Are the following statements about the cell cycle 'True' or 'False'?

a) (2 points) Variations in cyclin-cdk activity through the cell cycle are primarily due to changes in cdk levels

False

b) (2 points) cdk-activating kinase (CAK) is itself a cyclin-dependent kinase

True

c) (2 points) In yeast cells, the SCF complex binds to and degrades the inhibitor Sic1 to enable entry into S-phase

False

d) (2 points) The metaphase to anaphase transition is triggered by the degradation of separase

False

e) (2 points) Cdc14, a key regulator of exit from mitosis, is a protein kinase

False

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9) (6 points) In the cell fusion experiment where a cell in G2 was fused to a cell in S-phase, what happened to the G2 nucleus in the heterokaryon? Explain the molecular basis of this observation.

The G2 nucleus does not replicate its DNA (does nothing)

The G2 nucleus has already replicated its DNA. DNA replication results in the disassembly of the pre-replication complexes and the degradation of Cdc6.

Since there are no pre-replication complexes on the replication origins and because Cdc6 is not available from the cytoplasm of either cell in the fusion experiment, the G2 nucleus is unable to replicate its DNA.

10) (6 points) DNA damage results in phosphorylation of the p53 protein. How does this lead to cell-cycle arrest?

Phosphorylation dissociates p53 from Mdm2 and hence stabilizes it.

p53 cause an increase in the transcription of p21 (or Cip1).

p21 (Cip1) binds to and inhibits cyclin-cdk complexes.

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11) (6 points). Are the following statements about apoptosis True or False?

a) (2 points) Cells swell and burst open during apoptosis

False

b) (2 points) Caspase 8 binds directly to the Fas trimer

False

c) (2 points) In the C. elegans cell death pathway, the Ced-9 gene directly activates Ced-3 without involvement of the mitochondria

False

12) (8 points) In the lecture on G1 control, I told the class that each student should try to solve the following problem: Arthur Pardee showed that either phosphate deprivation or amino-acid starvation caused a cell-cycle arrest at exactly the same point in G1 (the restriction point). Outline the principles of a simple experiment that could test whether this is the case (i.e. test whether the two mechanisms of arrest stop the cell cycle at the same or different places in G1). (Hint: All you need to measure is radioactive thymidine incorporation to determine whether DNA synthesis has occurred). Hopefully you have thought about this question over the last couple of weeks and know the answer.

1) Experiment 1: Put the cells in medium depleted only of phosphate. Wait until all the cells have arrested. Then transfer them to medium that has phosphate but no amino acids. Measure thymidine incorporation after transfer to new medium.

2) Experiment 2: Put the cells in medium depleted of only of amino acids. Wait until all the cells have arrested. Then transfer them to medium that has amino acids but no phosphate. Measure thymidine incorporation after transfer to new medium.

If no thymidine incoporation is observed in either experiment, this means that the cells arrest at the same point in G1 whether they are depleted of amino acids or phosphates.