Problem Set #4
7.06 - Spring 2015

Name _____

Section _____

Question 1

A. You want to measure the activity of different signaling pathways. For each of the following assays, circle the pathways that would show a ***change*** for at least one protein in the pathway following pathway stimulation (aka ligand addition). Assume that this occurs for the basic pathway in the absence of any feedback, etc. and that you would be able to observe such a change even if it is transient. (0.5 pts each)

Plasma membrane localization

GPCR (light receptor in neurons) Wnt

TGF Beta Notch/Delta

Cytokine Receptor

Nuclear localization

GPCR (light receptor in neurons) Wnt

TGF Beta Notch/Delta

Cytokine Receptor

Phosphorylation state

GPCR (light receptor in neurons) Wnt

TGF Beta Notch/Delta

Cytokine Receptor

Presence of a full-length protein

GPCR (light receptor in neurons) Wnt

TGF Beta Notch/Delta

Cytokine Receptor

Question 2
Which of the following signal transduction events would be rapidly reversible (i.e., seconds, not minutes or hours)? In each case, if this event is rapidly reversible, indicate the most direct mechanism for this reversibility.

A. Binding of a ligand to a G protein coupled receptor

B. Activation of a heterotrimeric G protein

C. Phosphorylation of STAT by Jak kinase

D. Ubiquitination of a cytokine receptor by the E3 SOCS

E. Degradation of ubiquitinated I-kB by the proteasome

F. Methylation of a histone

G. Activation of an initiator caspase

H. Localization of Notch to the nucleus following activation of the Notch/Delta signaling pathway

Question 3
Which of the following would have a ***significant*** effect on the ability of a signaling pathway to induce its downstream effects? Briefly explain **why** this is the case, and what the effect would be (if present). If it is not possible to determine whether this would cause an effect, indicate why. (3 pts each)

A. Reduction in the number of cell surface receptors by 25%.

B. A change in the affinity of a receptor for its ligand from 10 μM to 10 nM. For these experiments, you are adding a ligand concentration that is limiting for the wild-type protein.

C. The kinase domain of a receptor tyrosine kinase is switched for a serine/threonine kinase. Ligand binding still results in kinase activation and trans-phosphorylation.

D. For the NF-KB pathway, overexpression of a general Ubiquitin deconjugating enzyme.

Question 4

You have identified a new signaling pathway in which addition of a ligand results in gene expression. Based on a series of approaches, you identify 5 different proteins. For one of these (Protein E), you generate a phospho-specific antibody that can monitor its phosphorylation state quantitatively using ELISA (think about this as a quantitative Western blot). You also generate a GFP reporter construct to quantitatively monitor gene expression. RNAi is a method to reduce or eliminate protein levels. Using these tools in combination with RNAi against the different components of the pathway, you obtain the following data.

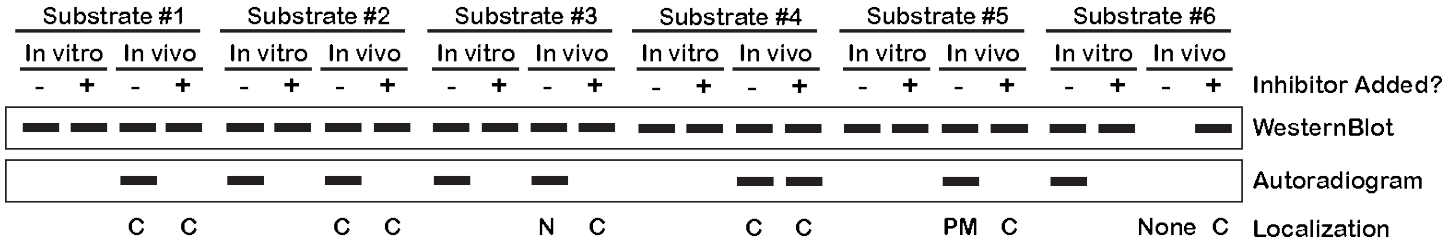
	Protein E Phospho-Ab		**GFP Reporter**	
	- Ligand	+ Ligand	- Ligand	+ Ligand
Control	10	200	5	500
Protein A RNAi	8	225	6	10
Protein B RNAi	155	185	465	490
Protein C RNAi	8	13	9	4
Protein D RNAi	7	190	413	485

Based on this data, provide an order for the pathway. If you are not able to order a given protein in the pathway relative to another, indicate this in your diagram. Use $\Rightarrow$ and $\perp$ symbols as appropriate.

Ligand addition **Protein E Phosphorylation** **Gene expression**

Question 5

You are working with a newly identified kinase that is required for the response to a growth hormone. You have generated purified kinase and a small molecule that specifically inhibits this kinase in vitro and in vivo. Using a genetic screen for proteins functioning in the same pathway, you have also identified 6 possible substrates for this kinase. For each of these potential substrates, you are able to monitor their protein levels (by Western blotting with antibodies against the potential substrates), the incorporation of radioactive ^{32}P-ATP (by Autoradiogram), and test the localization of the protein within the cell (using a GFP fusion and fluorescent microscopy). You conduct the experiments shown below. In each case, you conduct an in vivo experiment using intact cells (under conditions where this signaling pathway is activated and where you have added radioactive ATP to the cell), and a second experiment using an in vitro biochemical reaction where only the kinase, substrate, and radioactive ATP are present.



C = Cytoplasm, N = Nucleus, PM = Plasma Membrane

Based on these data, for each potential substrate, state whether this protein is a direct target of the kinase. In each case, also describe the effect of pathway activation on the protein.

Substrate #1:

Substrate #2:

Substrate #3:

Substrate #4:

Substrate #5:

Substrate #6:

Question 6

[Answering this question will require material from lecture 10]

You are interested in recovering Drosophila mutants with defects in the formation of the body. You do a genetic screen and recover a mutant in which the anterior and posterior ends of the embryo are missing. Drosophila is diploid and severe patterning defects will cause lethality, so you maintain this fly stock as a heterozygote. In the mutant stock, 25% of the embryos die and lack termini.

A. You are able to clone the gene mutated in this strain, and you find that it encodes an RTK receptor, and the mutant has an amino acid substitution of M to K in the predicted active site. Explain in genetic terms why this mutation gives the phenotype and numbers observed in the mutant stock.

B. What do you expect to be the phenotype for a mutation in the gene encoding SOS? A mutation in Raf?

C. Where in the embryo do you expect this RTK pathway to be active? You cannot biochemically measure RTK activity in only one part of the embryo, so how could you test where it is active?

D. What are two ways that activation of this RTK pathway could be spatially restricted in the embryo?

E. What might be the phenotype for a loss-of-function mutation in the GAP for Ras? Would you have trouble recovering this mutant? Why?