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

Yes.
Release of the ligand.

B. Activation of a heterotrimeric G protein

Yes.
GTP Hydrolysis (stimulated by a GAP/Effector)

C. Phosphorylation of STAT by Jak kinase

Yes.
Dephosphorylation by phosphatase

D. Ubiquitination of a cytokine receptor by the E3 SOCS

Yes.
Deubiquintination by deconjugase

E. Degradation of ubiquitinated I-kB by the proteasome

No.

F. Methylation of a histone

Yes.
Demethylation by histone demethlylase

G. Activation of an initiator caspase

No.

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

No.

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%.

   This likely wouldn't have an effect. Deleting an entire copy of a receptor (heterozygous null) is recessive. Loss of 25% of the receptors wouldn't be an issue. The signal is amplified.

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.

   If the binding is better, the chance of the receptor binding to a ligand and signaling downstream is increased. Given the 3 orders of magnitude change and the limiting ligand, this would likely give much stronger pathway activation.

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.

   Even if the kinase is activated, phosphorylation of serine/threonine residues would not provide a platform of SH2 domain binding (etc.) as these are specific for phospho-tyrosines. Thus, this would prevent pathway activity.

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

   This would prevent I-kB Ubiqutination and degradation (wouldn't be targeted to the proteasome as efficiently). It would reduce overall pathway activation.

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

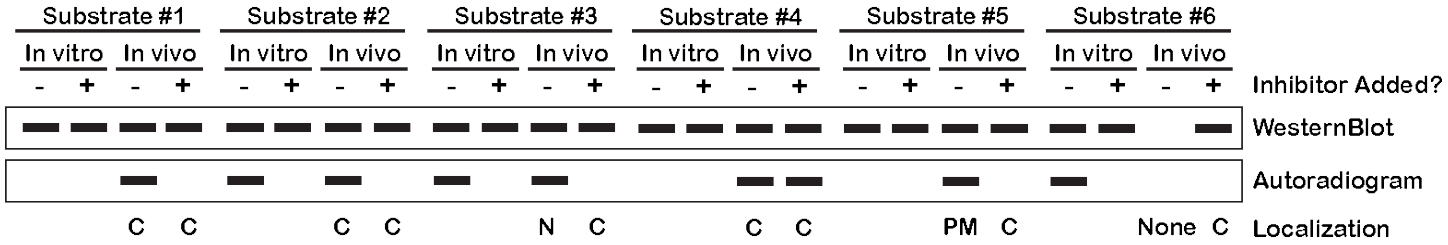
Based on this data, provide an order for the pathway. If you are not able to order a given protein in the pathway relative to another, indicate this in your diagram. Use ⇒ and ⊥ symbols as appropriate.

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

Protein A acts downstream of Protein E to promote (⇒) gene expression
Protein B acts upstream of Protein E to prevent (⊥) its phosphorylation
Protein C acts upstream of Protein E to promote (⇒) its phosphorylation
Protein D acts downstream of Protein E to inhibit (⊥) gene expression

It is not possible to definitively order protein A/D and B/C. If an order is given, it would be necessary to specify two possibilities (different combinations of activation and inhibition), but an order between these is not strictly necessary.

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:

Not a direct target.
Pathway activation causes phosphorylation of protein downstream of a kinase.

Substrate #2:

Direct target.
Pathway activation causes kinase to phosphorylate.
May activate protein, but no change in localization.

Substrate #3:

Direct target.
Phosphorylation of protein by kinase causes it to target to the nucleus.

Substrate #4:

Not a direct target.
Either not in pathway or upstream of kinase.

Substrate #5:

Not a direct target.
Phosphorylation of a different substrate by kinase causes this substrate to by targeted to the plasma membrane (such as by an SH2 domain)

Substrate #6:

Direct target.
Phosphorylation by kinase causes it to be targeted for degradation.

Question 6

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.

RTK signaling is required to form the termini of the embryo. The mutation recovered causes loss of catalytic activity of the kinase and is recessive, explaining why the phenotype is observed in only 25% of the embryos, those that are homozygous for the mutation.

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

Assuming the mutations are loss of function, they would cause 25% of the embryos to lack termini, because SOS and Raf are both downstream of RTK and required for signaling.

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?

RTK signaling would have to be occurring at the termini. You could test whether this was true by looking for nuclear localization of MAPK, a downstream effector in the RTK pathway. You could do this by fixing the embryos and staining them with an antibody against MAPK and a fluorescently labeled secondary antibody.

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

Either the RTK receptor is present only in the termini of the embryo, or more likely, its ligand is present only in these regions.

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?

Because GAP is required to inactivate Ras, a loss-of -function mutation in the GAP gene would lead to constitutive activation of the RTK pathway. This should

cause all parts of the embryo to adopt terminal fates. It would be difficult to recover because it is predicted to be dominant and would result in embryonic lethality.