QUESTIONS Cell signaling part 2

1 Antibodies are Y-shaped proteins that carry two identical sites that bind specific proteins.
1A) What do you think would happen if an antibody was added in the medium of cells cultured in vitro, assuming the antibody recognizes a portion of the extracellular region of a RTK expressed at the plasma membrane of these cells: the antibody would activate the receptor / inactivate the receptor / cause no change in receptor activity?

1B) How about an antibody that recognizes the kinase domain of the receptor?

2 "yes" or "no"? If no, explain what is correct.

1. An extracellular signaling molecule binds and activates a G-protein.
2. The activated GPCRs cause Gα to separate from Gβ and Gγ.
3. Adenylate cyclase produces cyclic AMP.
4. cAMP is produced from ADP.
5. cAMP activates protein kinase C.
6. Protein kinase A phosphorylates target proteins.

3 "yes" or "no"? If no, explain what is correct.

1. RAS is a kinase that phosphorylates the RAF MAP kinase.
2. EGF-R is a tumor suppressor gene.

4 Activated GPCRs activate G proteins by reducing the strength of binding of GDP to the α subunit of the G protein, allowing GDP to dissociate and GTP (which is present at much higher concentrations in the cell than GDP) to bind in its place. How would the activity of a G protein be affected by a mutation that reduces the affinity of the α subunit for GDP without significantly changing its affinity for GTP?

5 A single amino acid substitution on KRAS can eliminate its capacity to hydrolyze GTP, even in the presence of a GAP (GTPase-activating protein). Many pancreatic tumors (and other tumors) contain such a mutant KRAS protein expressed in the tumor cells. This mutant protein is called an oncoprotein, because it promotes tumor development. You have identified a new drug, which can block the dimerization of a receptor tyrosine kinase (RTK) that signals in cells *via* KRAS. Do you think your treatment will be effective against KRAS mutant pancreatic cancer, or not? Why?

6 Cytoplasmic Na^+ is much more abundant than Ca^{++} (10^{-3} vs 10^{-7} M). Propose why do you think cells use Ca^{++} and not Na^+ as secondary messenger for multiple signaling pathways?

7 Part of the cellular response to an RTK signaling pathway is dependent on effector protein A. Protein A changes conformation and becomes active if it is phosphorylated on a tyrosine by MAP kinase (ERK).

A) What would happen to a cellular signal if you mutate the tyrosine to 1) An amino acid that prevents the conformational change and 2) locks the protein in the active state?

B) What major signaling proteins are upstream of this signaling cascade activating Protein A?

C) What other signaling pathways could be activated by the RTK? What protein structures have to be present on the RTK in order to activate the signaling pathway?

8) Decide whether each of these statements is true or false, and then explain why.
A) Different isoforms of protein kinase A in diferent cell types explain why the efects of cyclic AMP vary depending on the target cell.

B) The activity of any protein regulated by phosphorylation depends on the balance at any instant between the activities of the kinases that phospho- rylate it and the phosphatases that dephosphorylate it.

C) Most intracellular signaling pathways provide multiple opportunities for amplifying a response to an extracellular signal.

9)
What is “cyclic” about cyclic AMP?

10)
CaM-kinase II is a remarkable molecular memory device. How does CaM-kinase II “remember” its exposure to Ca2+/calmodulin and why does it eventually “forget”?

11)
The Ras protein functions as a molecular switch that is turned on by a guanine nucleotide exchange factor (GEF) that causes it to bind GTP. A GTPase-activating protein (GAP) turns the switch of by inducing Ras to hydrolyze its bound GTP to GDP much more rapidly than in the absence of the GAP. hus, Ras works like a light switch that one person turns on and another turns of. In a cell line that lacks the Ras-speciic GAP, what abnormalities in Ras activity, if any, would you expect to ind in the absence of extracellular signals, and in their presence?

12)
MAP kinase kinase kinase (MAPKKK) activates MAP kinase kinase (MAPKK) by phosphorylation of two serine side chains. Doubly phos- phorylated (active) MAPKK, in turn, activates MAP kinase (MAPK) by the phosphorylation of a threonine and a tyrosine. he doubly phospho- rylated MAPK then phosphorylates a variety of target proteins to bring about complex changes in cell behavior. It is possible to write down all of the rate equations for the individual steps in this activation cascade, as well as for the removal of the phosphates (inactivation) by protein phos- phatase, and to solve them by making reasonable assumptions about the concentrations of the

proteins. he calculated plot of activation of the kinases versus input stimulus is shown in Figure 15–16. Why is the very steep response curve for MAPK a good thing for this signaling pathway?

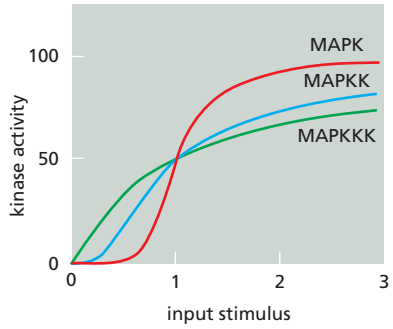

Figure 15–16 Stimulus–response curves for the components of the MAPK cascade (Problem 15–99). For ease of comparison, the curves have been normalized so that an input stimulus of 1 gives 50% activation of the kinases.