BIO/CBIO 3400
Fall 2012
First Quiz
Fechheimer/Lechtrek

QUESTION 1 (25 POINTS)

(a) (15 POINTS) Compare and contrast the mechanisms of signal transduction for adrenaline and light. Be sure to include structural information regarding key elements of the pathway.

	Adrenaline signaling	Light signaling	
Location	Adipocyte	Rod cell of retina	(-1 pt)
Stimulus	Adrenaline	Photon of light	(-1 pt)
Receptor	Adrenaline receptor	Rhodopsin	(-1 pt)
Transducer	Gs protein	Transducin	(-1 pt)
Target	Adenyl cyclase	cGMP PDE	(-2 pts)
2nd messenger	Increase in cAMP	Decrease in cGMP	(-2 pts)
Result	PKA is activated (Fight or flight response)	Sodium channel is closed (Vision)	(-2 pts)

Receptor is a 7 TM integral membrane protein (GPCR). (-1 pt)

G protein is a heterotrimer with alpha, beta, and gamma subunits. alpha binds GTP. (-1 pt)

When alpha gets GTP, it dissociates from beta/gamma. (-1 pt)

G protein is a key player in both pathways. Without the stimulus, alpha subunit of the G protein binds to GDP as an inactive form. When a receptor receives stimulus, alpha subunit binds to GTP (receptor as a GEF) and is active. Meanwhile, the active alpha subunit is released from beta/gamma subunits to activate downstream targets.
(-2 pts)

When the signaling needs to be turned off, GAP is utilized to activate the GTPase activity of alpha subunit. As a result, alpha subunit hydrolyzes its own GTP to GDP and becomes inactive. (-1 pts)

(b) (10 points). Describe how an action potential is propagated along a membrane. For one named channel, give a detailed account of the structure of the channel, how the structure contributes to the process, and how regulation of the process occurs.

Mediated by voltage-gated sodium channel. (-1 pt)

Voltage-gated sodium channel has 3 states: Closed, open, and inactivated.

Action potential travels uni-directionally. When action potential comes in, the channel is opened by the change of membrane potential (depolarization). Soon after that, channel will be inactivated (refractory period) in order to prevent backward propagation. (-3 pts)

Voltage-gated sodium channel:

Pore: 24 transmembrane spans. 4 set of 6 in a single peptide. (-1 pt)

Voltage sensor: + charges M4 (-1 pt)

Channel inactivation: IFM motif (-1 pt)

Ion selectivity: the loop between M5 & M6 is inserted in the lipid bilayer and lines the pore. The selectivity loop determines sodium ions can pass the channel. The side chains of amino acids in the loop interact with hydrophobic core and set the diameter of pore, and carbonyl groups interact with sodium ions to strip water.
(-3 pts)

2. (25 POINTS)
(a) (14 Points) Describe the series of events that occur during signaling by a receptor tyrosine kinase pathway, beginning with addition of a ligand to cells. Use the outline provided below to organize your answer, by naming and describing the functions and interactions of at least seven different proteins that participate in the pathway.

i. Receptor tyrosine kinase. When ligands bind to RTK on the cell membrane, RTK will dimerize and auto-phosphorylate tyrosine residues (trans-phosphorylation). Now RTK is being activated. (-2 pts)

ii. Grb2. It is an adaptor protein connecting phospho-tyrosine on RTK (through one SH2 domain) and proline-rich region on SOS (through two SH3 domains) when RTK is activated. (-2 pts)

iii. SOS. It is a Ras-GEF. When RTK is activated, SOS binds to SH3 domains of Grb2 and stimulates the activity of Ras to exchange its GDP to GTP. (-2 pts)

iv. Ras. It is a peripheral, membrane-bound G protein. When RTK is activated, Ras-GDP is switched to Ras-GTP as an active form. Ras-GTP will activate downstream signaling components. (-2 pts)

v. RAF. It is a kinase and also known as MAPKKK. 14-3-3 binds to inactive RAF. Ras-GTP replaces 14-3-3 and activates RAF. (-2 pts)

vi. MEK. It is a kinase and also known as MAPKK. Active RAF phosphorylates and activates MEK. (-2 pts)

vii. ERK. It is a kinase and also known as MAPK. MEK phosphorylates and activates ERK to regulate protein activities or gene expressions. (-2 pts)

(b) (11 POINTS) Identify the following and describe their interactions, regulation, and function.

i. IP_3: Inositol 1, 4, 5-triphosphate. PLC-beta (GPCR signaling) and PLC-gamma (RTK signaling) cleave PIP_2 to make DAG and IP_3. IP_3 binds to calcium channels on ER membrane to turn on the releasing of calcium ions into cytoplasm from ER lumen. Calcium ion can work together with DAG to activate PKC. (-3 pts)

ii. TOR: A kinase, Target of Rapamycin. When PI3K is activated by RTK, it phosphorylates $PI(4,5)P_2$ to $PI(1,4,5)P_3$ on the cell membrane. Akt (PKB) binds to $PI(1,4,5)P_3$ and is activated by both PDK1 (also binds to $PI(1,4,5)P_3$) and mTOR (complex 2). Active Akt phosphorylates Bad to release apoptosis inhibitory protein in order to inhibit apoptosis. Active Akt can also inactivate Tsc (a GAP of Rheb). Rheb-GTP can further activate mTOR complex 1 to promote cell growth. (-3 pts)

iii. BARK: Beta-adrenergic receptor kinase, a GPCR kinase (GRK) of adrenaline receptor. It functions to turn off the adrenaline signaling by phosphorylating the cytoplasmic region of adrenaline receptor. An active adrenaline receptor will stimulate BARK to phosphorylate the receptor. When the receptor is phosphorylated, beta-arrestin will recognize the phosphorylated receptor and bind to it to turn off the adrenaline signaling. (-3 pts)

iv. PTEN: A phosphatase that remove a phosphate from carbon 3 on PI(3)P, $PI(3,4)P_2$, or $PI(3,4,5)P_3$. It serves as a negative regulator of PI3K signaling pathway to suppress tumor growth. (-2 pts)