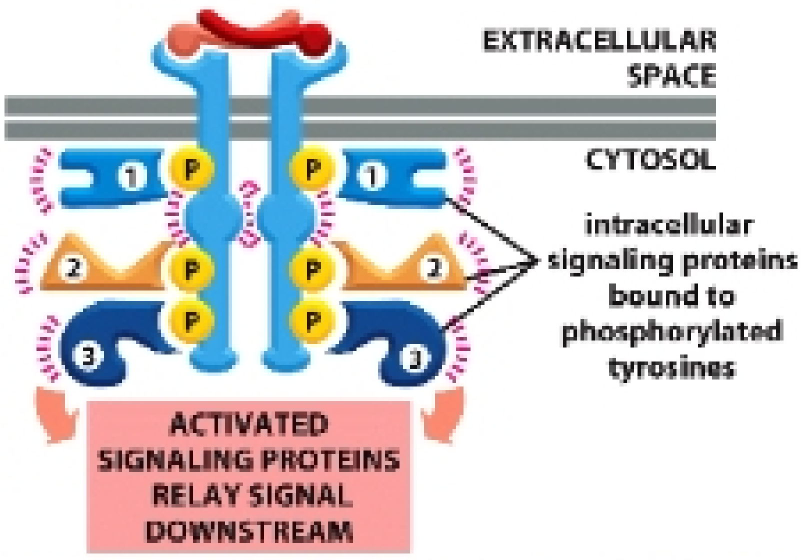
CBIO 3400 – Week 3 Study Questions

1. Compare and contrast how GPCR and RTK utilize nucleotide triphosphates as the energy source to turn on downstream signaling pathways.

   RTKs use nucleotide triphosphates to auto-phosphorylate and then trans-phosphorylate themselves to activate. Once activated, RTKs assemble large, multi-molecular complexes that create a signaling cascade; the proteins that make up this large complex relay the signal downstream.



   GPCRs use nucleotide triphosphates to activate their α and βγ complexes, both of which can regulate the activity of target proteins in the plasma membrane. These activated complexes bind and activate an enzyme, such as adenylyl cyclase, which causes the production of a second messenger, such as cAMP, that can bind to and activate a target, such as Protein Kinase A.

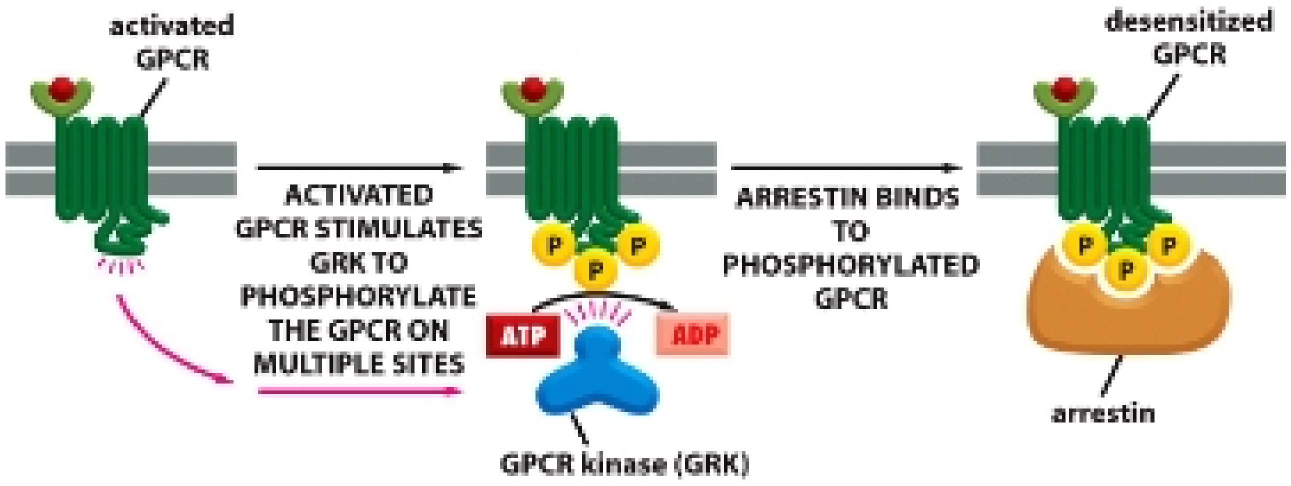
2. Use one signaling pathway as an example to explain how RTK and G-protein are associated in a single signaling pathway.

   The signaling pathway in the fly eye uses both RTK and G-protein to transmit a signal...?

3. What mechanisms exist to down-regulate (turn off) signaling from GPCR?

A GPCR can turn itself off with GAP (GTPase activating protein), which stimulates GTPase by the activated subunit (such as α) and causes phosphodiesterase to cleave cAMP. This causes the protein kinase to turn off and effectively turns off GPCR signaling with the reassociation of the α and βγ subunits on the G protein.

A GPCR can also turn itself off by being phosphorylated at multiple sites, attracting a protein, such as arrestin, and desensitizing the GPCR.



4. Compare and contrast signaling through Rhodopsin versus signaling through the β-adrenergic receptor.

Adrenalin Pathway – β-Adrenergic Receptor

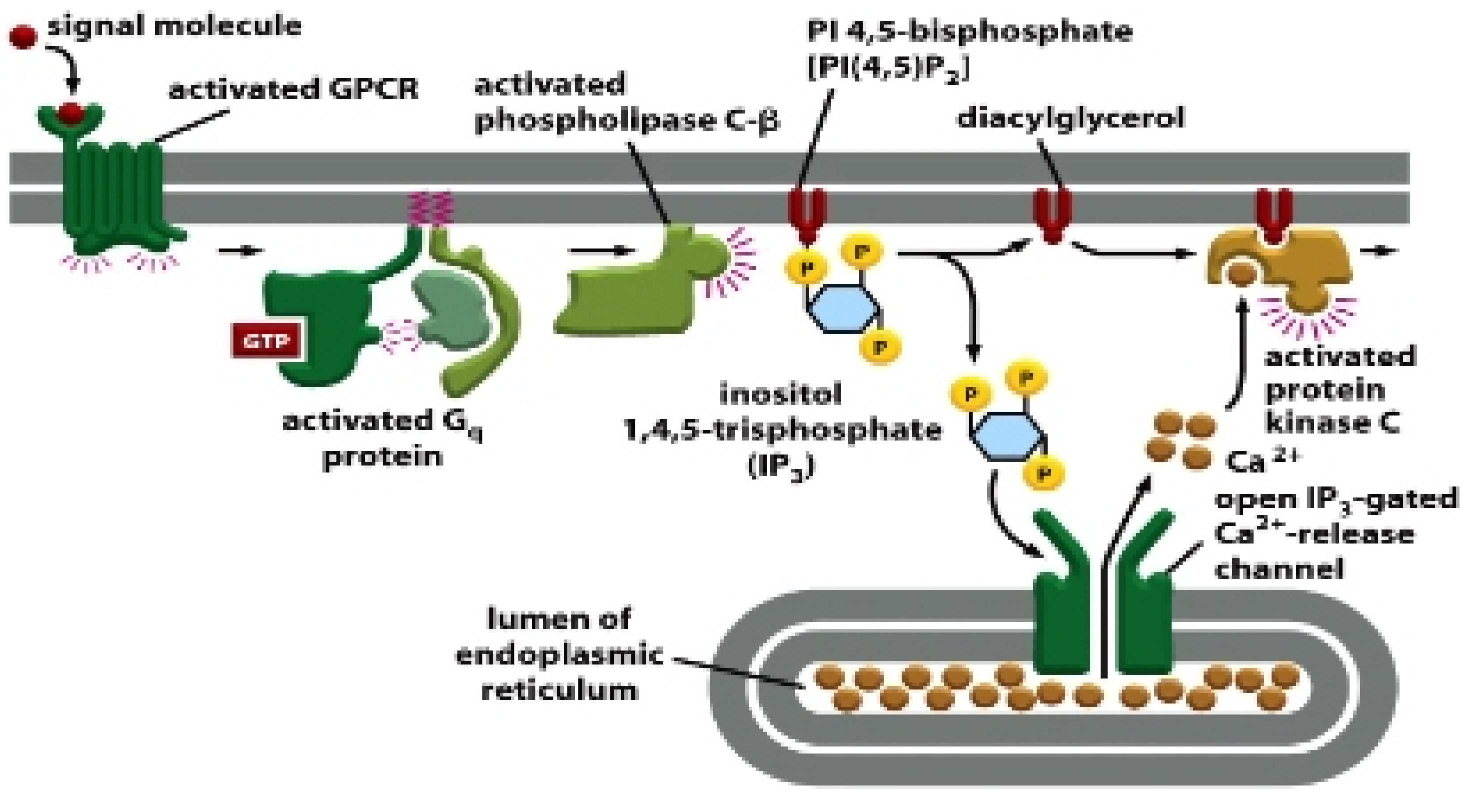
The signal adrenalin binds to the β-adrenergic receptor. This causes the α and βγ subunits to dissociate, at which point the α_s subunit binds to and turns on the enzyme adenylyl cyclase. Adenylyl cyclases activates the second messenger cAMP, freeing the catalytic units. cAMP then binds to Protein Kinase A.

G Protein Signaling in the Retina – Rhodopsin

Light is the signal that activates the receptor rhodopsin. This activates the G protein transducin, which stimulates a cGMP phosphodiesterase. The cGMP phosphodiesterase cleaves cGMP, causing a decrease in cGMP. This decrease is effectively the second messenger because cGMP is the ligand that activates ligand-gated Na^+ channel. The decrease in cGMP causes the pathway to reach its target: closing of the Na^+ channel.

5. IP_3 plays an important role in Calcium-dependent signaling. Explain where and how IP_3 is synthesized and how it is related to Calcium-dependent signaling.

IP_3 is synthesized in the cytosol when phospholipase C-β cleaves PIP_2 to produce diacylglycerol (DAG) and inositol triphosphate (IP_3). IP_3 is an important part of Ca^{2+}-dependent signaling because IP_3 can travel a much longer distance in the cytosol than Ca^{2+} can. IP_3 travels to the lumen of the endoplasmic reticulum, where it binds and opens an IP_3-gated Ca^{2+} release channel. The Ca^{2+} can then travel a shorter distance to bind and activate Protein Kinase C. *See related image on next page.*



6. Describe the differences in function, protein interactions, and regulation of Protein Kinase A, Protein Kinase B, and Protein Kinase C.

Protein Kinase A

Function – phosphorylates specific serines and threonines on selected target proteins, including intracellular signaling proteins and effector proteins, thereby regulating their activity

Protein Interactions – consists of a complex of 2 catalytic subunits and 2 regulatory subunits → binding of cAMP to regulatory subunits alters their conformation, causing them to dissociate from the complex → the released catalytic subunits are thereby activated to phosphorylate specific target proteins

Regulation – in unstimulated cells, the phosphodiesterase keeps the local cAMP concentration low, so bound PKA is inactive; in stimulated cells, cAMP concentrations rapidly rise, overwhelming the phosphodiesterase and activating the PKA

Protein Kinase B (also called Akt)

Function – regulates cell survival and metabolism by binding and regulating many downstream effectors; promotes cell growth and survival

Protein Interactions – PKB activates mTOR to promote cell growth

Regulation – involved in the PI-3-K/PKB/mTOR pathway; activation requires binding of the PH domain to 3-phosphoinositides and phosphorylation of PKB by PDK1