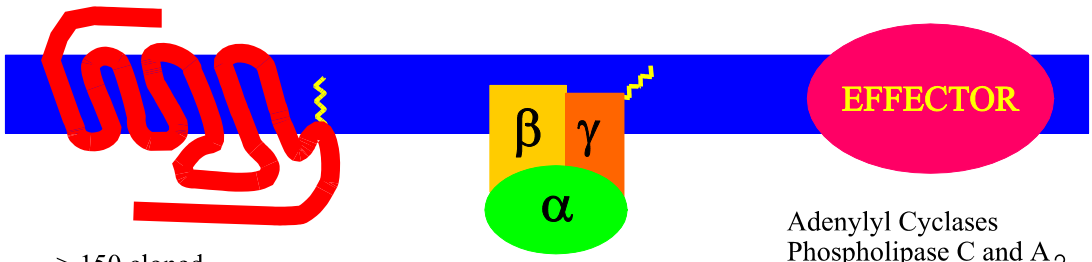
TOPIC 1:

A) REGULATION OF G PROTEIN-COUPLED RECEPTOR (GPCR) ACTIVITY

B) COUPLING OF GPCRS TO NON-G PROTEIN-LINKED SIGNAL TRANSDUCTION PATHWAYS.

GPCR Topology

G protein-coupled receptors (GPCRs) are seven transmembrane spanning receptors that are also referred to as serpentine or (inappropriately) as heptahelical receptors. The transmembranes are composed of protein helices and are linked by intracellular and extracellular loop domains. Coupling of most G protein-coupled receptors to heterotrimeric G proteins involves the third intracellular loop and the proximal region of the carboxyl-terminal tail. However, in the case of metabotropic glutamate receptors that are expressed in the brain the second intracellular loop rather than the third intracellular loop is required for G protein coupling



> 150 cloned
Adrenergic
Dopamine
Muscarinic
Serotonergic
Other Small Molecules
Glycoprotein Hormones
Peptides
Sensory

Mammalian, Avian
Fly, Yeast, Slime Mold

16 + α subunits
6 + β subunits
9 + γ subunits

Stimulatory or
Inhibitory

Sensitivity to
Bacteria Toxins

Adenylyl Cyclases
Phospholipase C and A_2
cGMP phosphodiesterase
Potassium Channels
Calcium Channels
Others ?

Figure 1: Basic components of a G protein-coupled receptor signal transduction complex.

GPCR Ligand Binding

There are at least 4 distinct mechanisms by which GPCR agonists bind to and activate GPCRs. **1)** In the case of GPCRs that bind small molecule, such as neurotransmitters and small peptide agonists and antagonists bind within a hydrophobic ligand pocket formed by the seven transmembrane spanning domains. **2)** In the case of peptide or large glycoprotein hormone receptors agonist binding involves the extracellular loop domains and the amino-terminal tail of the receptor. **3)** Unlike observed for most receptors that bind small molecules, metabotropic glutamate receptor (mGluR) activation involves glutamate binding to a stretch of amino acids within the long extracellular amino-terminal tail. The structure of this domain resembles bacterial periplasmic binding proteins and the binding of ligand is akin to a Venus fly-trap model of ligand sequestration. **4)** Protease activated receptors such as the thrombin receptor encode their own tethered ligands within the amino terminal tail of the receptor that are cleaved by proteases.

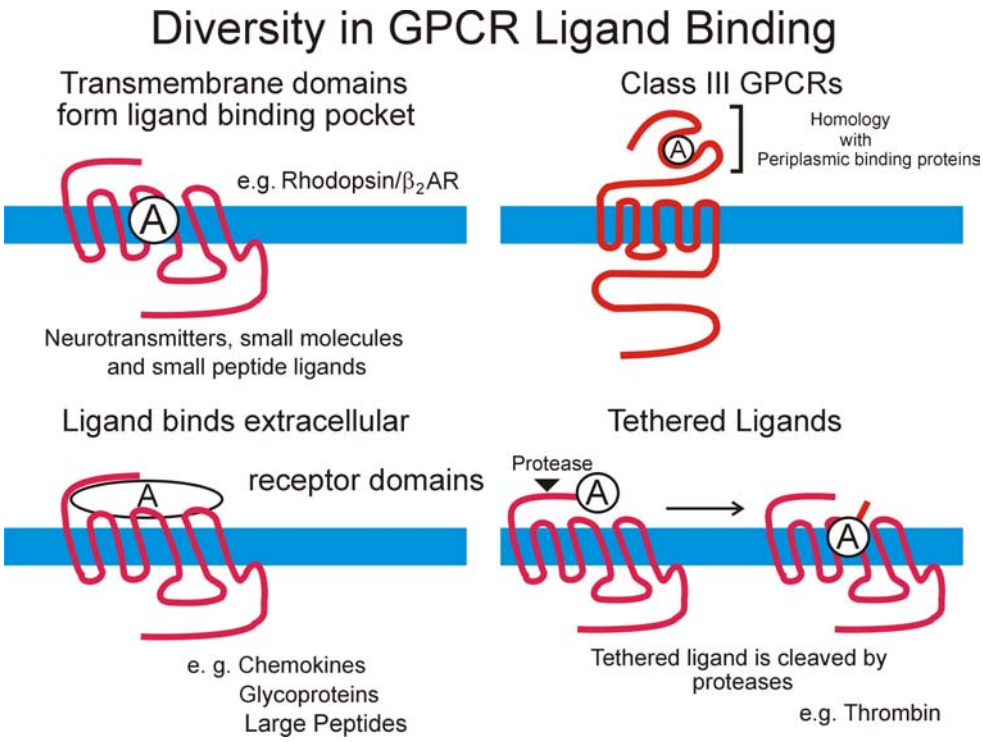


Figure 2. Models of GPCR ligand binding diversity

GPCR Agonist Binding Theory

The simplest description of the theory explaining GPCR activity states suggests that the GPCRs can spontaneously isomerize between inactive and activated receptor conformations leading to the stimulation of the exchange of GDP for GTP on the G protein α-subunit. Consequently, GPCRs exhibit some degree of spontaneous activity in the absence of agonist stimulation and receptor activation by agonists promotes the stabilization of the activated receptor conformation. The extent of spontaneous (intrinsic) activity differs from one receptor to the next. For example, β-adrenergic receptors exhibit low intrinsic activity whereas metabotropic glutamate receptors (mGluRs) exhibit profound intrinsic receptor activity. Several mutations in the amino acid sequences encoding GPCRs lead to increased spontaneous receptor activity that leads to diseases. Examples include precocious puberty (lutenizing hormone receptor overactivity), thyroid adenomas (thyroid stimulating hormone receptor overactivity) and retinitis pigmentosa (rhodopsin overactivity). Inverse agonists are drugs that do not stimulate receptor activation but rather selectively recognize or stabilize the inactive receptor conformation and thereby reduce the spontaneous or intrinsic activity of GPCRs. Full antagonists do not preferentially recognize or stabilize either the inactive or active receptor conformation. Thus, the treatment of cells with full antagonists will result in no net change in receptor activity. Rather, the antagonists will block the activity of both agonist and inverse agonists.

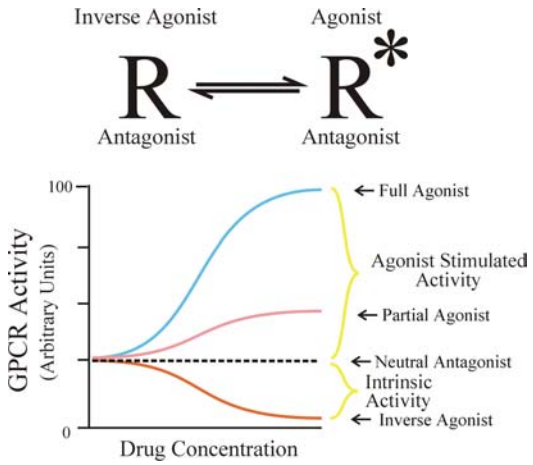


Figure 3. Schematic Model of GPCR Agonist Theory

G Protein Activation

The agonist activation of GPCRs stimulates the exchange of GDP for GTP on the heterotrimeric G protein α-subunit. The exchange of GDP for GTP serves to activate the heterotrimeric G proteins promoting the dissociation of the α- and βγ-subunits. In this respect, GPCRs act as guanine nucleotide exchange or releasing factors (GEFs or GRFs). Once dissociated both the α- and βγ-subunits are free to modulate the activity of effector enzymes (e.g. adenylyl cyclase and phospholipase C) and/or regulate the activity of ion channels (e.g. calcium and potassium channels). Depending upon the class of G proteins activated (see below) the enzyme and channel activity is either increased or decreased. Inactivation of G proteins occurs as the consequence of the hydrolysis of GTP to GDP on the α-subunit and is mediated by the intrinsic GTPase activity of the G protein. This allows for repetitive receptor-G protein coupling as well as the ability of multiple receptors to activate multiple G proteins. The intrinsic GTPase activity of heterotrimeric G proteins is accelerated by GTPase activating proteins (GAPs), such as regulators of G protein signaling (RGS proteins) and the effector enzymes activated by the G protein α-subunit (e.g. phospholipase C and adenylyl cyclase).

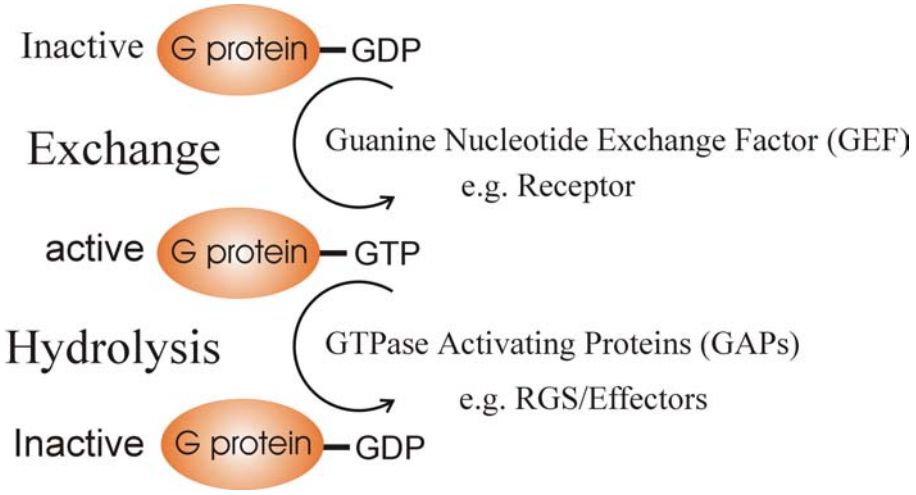


Figure 4. Schematic representation of the G protein activation/inactivation cascade

Effector Activation Inactivation by Different Heterotrimeric G proteins

For the purpose of the present course, we will focus on three major classes of heterotrimeric G proteins, Gs (stimulatory), Gi (inhibitor), and Gq. The activation of Gs-coupled GPCRs leads to the stimulation of the activity of downstream effector enzymes. The activation of Gi-coupled GPCRs antagonizes the activation of effector enzymes by Gs-coupled receptors and thus are considered inhibitory. Finally, Gq-coupled receptors stimulate the activity of phospholipase C resulting in the increased formation of intracellular diacylglycerol (DAG) and inositol 1, 4, 5, triphosphate (IP_3) concentrations. The increased IP_3 concentrations stimulate the release of calcium from intracellular stores by activating the IP_3 receptor localized on the endoplasmic reticulum (ER). As mentioned above, these G proteins also regulate ion channel activity, a property that will not be discussed.

Table 1: Examples of the effect of Activating different G protein isoforms

G Protein	Receptor	Effector	Consequence
Gs	e.g. β1-adrenergic receptor D1 Dopamine receptor mGluR2	Increased Adenylyl Cyclase activity	Increased PKA activity
Gi	e.g. D2 dopamine receptor mGluR4 opioid receptors	Decrease Adenylyl Cyclase Activity	Inhibition of PKA activity
Gq	e.g. angiotensin II receptor mGluR1 mGluR5	Phospholipase C Activation	Increased IP3 Calcium DAG

Patterning of GPCR Signaling

The activation of GPCRs is generally considered to result in tonic and/or transient increases in effector enzyme activity. However, in the case of some GPCRs, such as mGluRs, persistent receptor activation results in the patterning of effector enzyme responses. For example, the activation of both mGluR1 and mGluR5 results in oscillatory G protein coupling, and oscillations in IP_3 formation, Ca^{2+} release from intracellular stores and protein kinase C (PKC) activity. This oscillatory patterning of glutamate receptor signaling is especially important in the processes that contribute to the development of the central nervous system such as the formation and elimination of synaptic contacts, as well as the regulation of memory and learning in the neonatal and adult brain. mGluR1 and mGluR5 activation stimulates oscillations in second messenger activity at different frequencies, these differences are translated in differences in protein kinase activity that may result in the differential regulation of transcription factors such as NF-κB and NFAT.

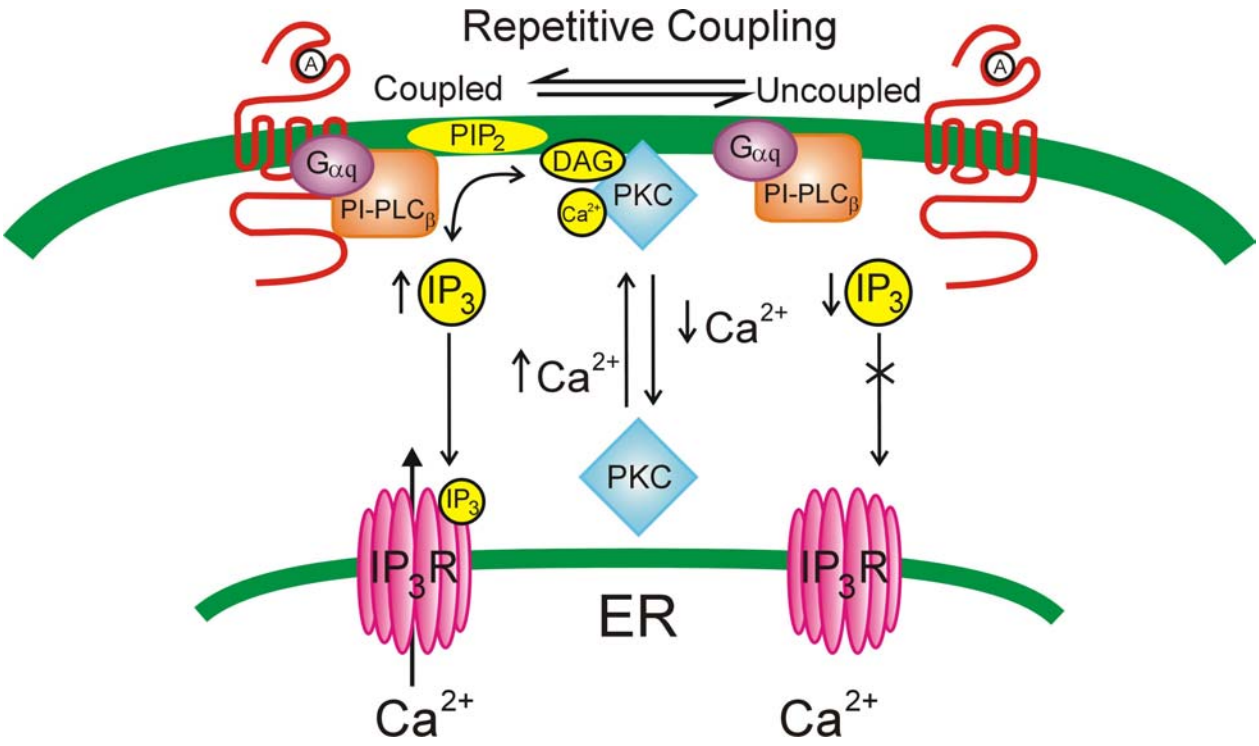


Figure 5. Schematic model of the events required for oscillatory mGluR signaling

Receptor Desensitization and Heterotrimeric G protein uncoupling:
For detailed overview see the review article provided:
Ferguson, S. S. G. (2001) Evolving concepts in G protein-coupled receptor endocytosis: The role in receptor desensitization and signaling. ***Pharmacol. Rev,*** 53, 1-24.

Receptor Inactivation: Inactivation of G protein-coupled receptor responsiveness is mediated by the covalent modification (**phosphorylation**) of intracellular receptor serine residues by both second messenger-dependent protein kinases (e.g. PKA, PKC) and **G protein-coupled receptor kinases (GRKs)**.

Second messenger-dependent protein kinase activation and desensitization: The activation of second messenger-dependent protein kinases such as PKA and PKC occurs as the consequence of receptor activation. The coupling of GPCRs via Gs to the activation of adenylyl cyclase leads to increased intracellular cAMP and the activation of the cAMP-dependent protein kinase (PKA). The coupling of receptors via Gq to the activation of PLC-β and increased IP3 and diacylglycerol formation results in the activation of protein kinase C (PKC). The second messenger-dependent protein kinases exhibit the capacity to phosphorylate both agonist-activated receptors and receptors that have not been exposed to agonist. This agonist-activation independent phosphorylation of GPCRs is termed heterologous receptor desensitization. The post-translational modification of the receptor due to PKA- or PKC-mediated phosphorylation inhibits the ability of the receptor to associate with heterotrimeric G proteins.

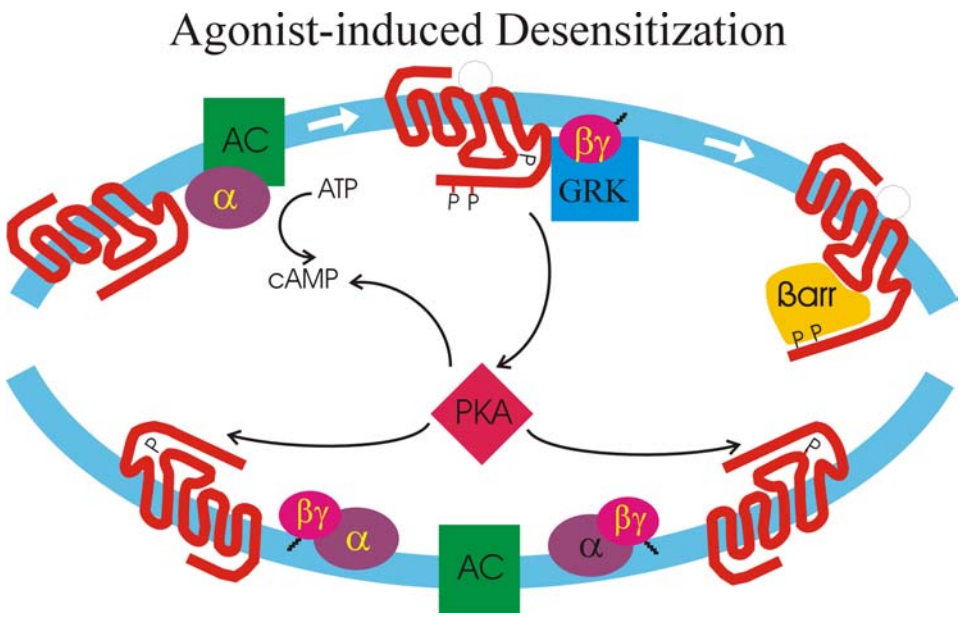


Figure 6: Schematic representation of homologous and heterologous receptor desensitization mechanism in a cell following the activation of Gs coupled GPCRs.

GRK-dependent phosphorylation and desensitization: GRKs specifically phosphorylate and desensitize agonist-activated GPCRs. The phosphorylation of agonist-activated receptors is termed homologous receptor desensitization and is mediated by both GRKs and second messenger-dependent protein kinases. GRKs are cytosolic proteins that are recruited to the plasma membrane in response to GPCR activation. Seven distinct GRK isoforms exist: GRK1-GRK7. There are mutiple mechanisms by which this is achieved. GRK1 and GRK7 are localized to the plasma membrane in response to GPCR activation as the consequence of the lipid modification (isoprenylation) of their C-terminal domains. In the case of the β-adrenergic receptor kinases (βARK1/2 or GRK2/3) plasma membrane targeting is mediated by the association of the carboxyl-terminal domain of the kinase with the βγ-subunit of the heterotrimeric G protein. GRK4 and GRK6 are palmitoylated resulting in the localization of these kinases to the plasma membrane in the absence of agonist activation. GRK5 has a polybasic carboxyl-terminal domain that associates with membrane lipid moieties. GRKs phosphorylate residues within the third intracellular loop and carboxyl-terminal domains of GPCRs.

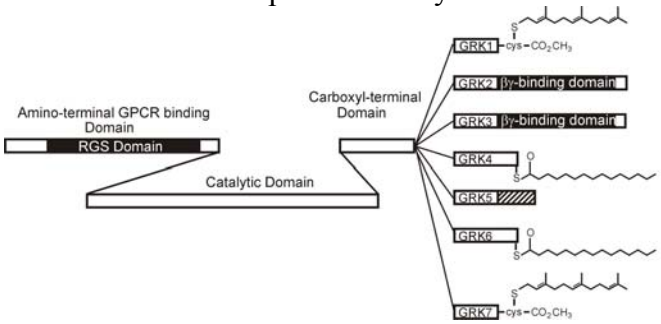


Figure 7. Schematic of GRK isoform structure

Arrestins: GRK receptor phosphorylation on its own is not sufficient for full receptor desensitization but rather increases the affinity of the receptor protein for **arrestin proteins** that sterically uncouple the receptor from heterotrimeric G proteins.

Receptor Endocytosis: The internalization of agonist-activated receptors serves to reduce the number of cell surface receptors that are available to become activated by agonist. Therefore, receptor internalization contributes to receptor desensitization by limiting the number of receptors at the cell surface available for agonist activation. However, in the case of many receptors, receptor internalization is required for receptor dephosphorylation and reactivation. Internalized GPCRs are targeted to the endosomal compartment of cells where a receptor-specific phosphatase dephosphorylates the receptor. Subsequently, the receptors are recycled back to the cell surface as fully function receptors. The mechanisms involved in targeting GPCRs for internalization are the same as those involved in receptor desensitization. GPCR endocytosis requires GRK phosphorylation followed by β-arrestin binding. β-Arrestins via their capacity to interact with both clathrin and the β-adaptin subunit of the AP2 adaptor complex (a complex involved in the formation of clathrin coated vesicles) target agonist-activated GPCRs to clathrin coated pits. These pits invaginate and form vesicular structures that pinch off from the cell surface and carry their receptor cargo to the endosomal membrane compartment of the cell. In the case of receptors like the β2-adrenergic receptor internalized receptors are dephosphorylated in the endosomes compartment of the cell and are recycled back to the plasma membrane surface as fully functional receptors.

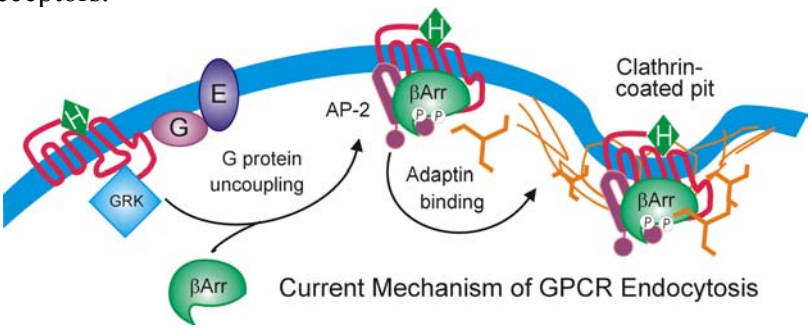


Figure 8: Events leading to the internalization of a GPCR

In response to agonist-stimulation many GPCRs are internalized but are not recycled back to the cell surface In some cases, internalized GPCRs, such as the protease-activated receptors (PAR) and endothelin B receptor, are predominantly targeted to lysosomes for degradation. In the case of protease-activated receptors, the carboxyl-terminal tail regulates targeting to lysosomes. For other GPCRs, such as the $AT_{1A}R$, internalized receptors are not necessarily sorted to lysosomes but may be retained within the endosomal compartment. As a consequence, by virtue of the fact that some receptors do not recycle, they will mediate transient responses to agonist. For example, endothelin A receptors are efficiently recycled and mediate persistent responses to endothelin, whereas the endothelin B receptors are targeted to lysosomes and mediate only transient responses to endothelin.

While β-arrestin-mediated internalization is well correlated with the inhibition of GPCR recycling, exceptions exist. For example, the neurokinin 1 receptor, which internalizes complexed with β-arrestin, is efficiently recycled back to the cell surface and is effectively resensitized. Therefore, multiple patterns of GPCR desensitization and resensitization exist. These include: 1) GPCRs that do not internalize in response to agonist, e.g. β_3-adrenergic receptor. 2) GPCRs that

are internalized without β-arrestin bound, and that are dephosphorylated and recycled back to the cell surface, e.g. β_2-adrenergic receptor. 3) GPCRs that are internalized with β-arrestin bound but that are dephosphorylated and rapidly recycle back to the cell surface following the dissociation of β-arrestins in the intracellular compartment of the cell, e.g. neurokinin 1 receptor. 4) GPCRs that are internalized with β-arrestin bound and are either retained in endosomes and/or targeted to lysosomes, e.g. $AT_{1A}R$ and PAR. Although β-arrestin appears to play a central role in regulating the intracellular trafficking properties of GPCR, it is likely that the manifestation of distinct intracellular trafficking properties also involves other components of the endocytic machinery.

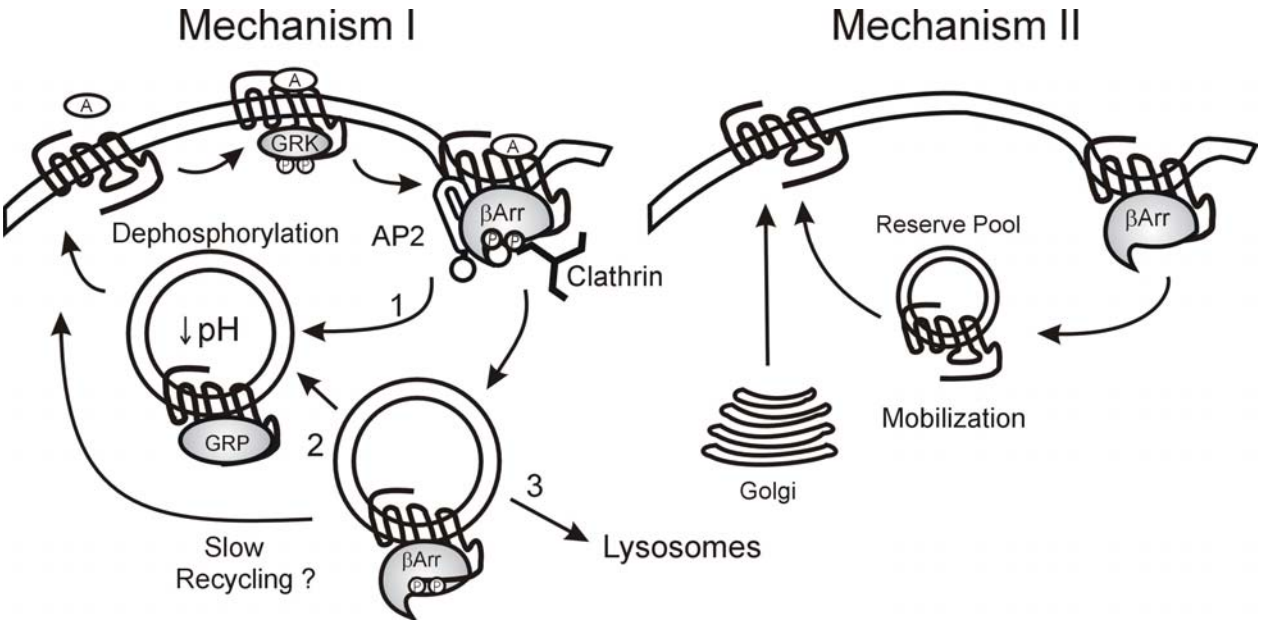


Figure 9: Schematic diagram showing the steps leading to the internalization and transport of a GPCR through the intracellular compartments of the cell.

Non G protein-linked GPCR signalling:

Role of β-Arrestins in GPCR-mediated Mitogenic Signaling

Both GPCRs and receptor tyrosine kinases (RTKs) stimulate mitogenesis in part via mitogen-activated protein (MAP) kinase cascades. The mechanisms by which GPCRs activate MAP kinase signal transduction cascades is poorly understood but appears to utilize the same intermediates as those activated by RTKs.

GPCR→ $G_{\beta\gamma}$→ *Tyr Kinase?* → Shc→ Grb2-mSos→ Ras→ Raf→ MEK→ MAPK

Recent studies with both RTKs and the β_2AR revealed that activation of MAPK requires the internalization of a macromolecular complex of proteins via clathrin-coated pits. In the case of the β_2AR, stimulation of MAPK phosphorylation could be blocked by both β-arrestin and dynamin dominant-negative inhibitors of clathrin-coated vesicle-mediated endocytosis. More recently, it was demonstrated that β-arrestins contributed directly to GPCR stimulated tyrosine kinase cell signaling their interaction with c-Src kinases. β-Arrestins bind to the SH3 domain of c-Src kinase, via an N-terminal polyproline motif. β-Arrestins both activate c-Src kinase activity and target the kinase to the plasma membrane bound receptor that in turn serves as a scaffold for

the formation of macromolecular complex of signalling proteins. Hence, β-Arrestins uncouple receptors from G protein-mediated signal transduction cascades and act as a molecular switch allowing GPCRs to function as RTKs, both as a consequence of their interaction with c-Src kinase and their function as endocytic adaptor proteins. The complex of GPCR/βarrestin/cSrc/Shc/Sos/Ras/Raf is formed at the plasma membrane and is internalized via clathrin-coated pits. Blockade of endocytosis prevents the phosphorylation of MEK and MAPK.

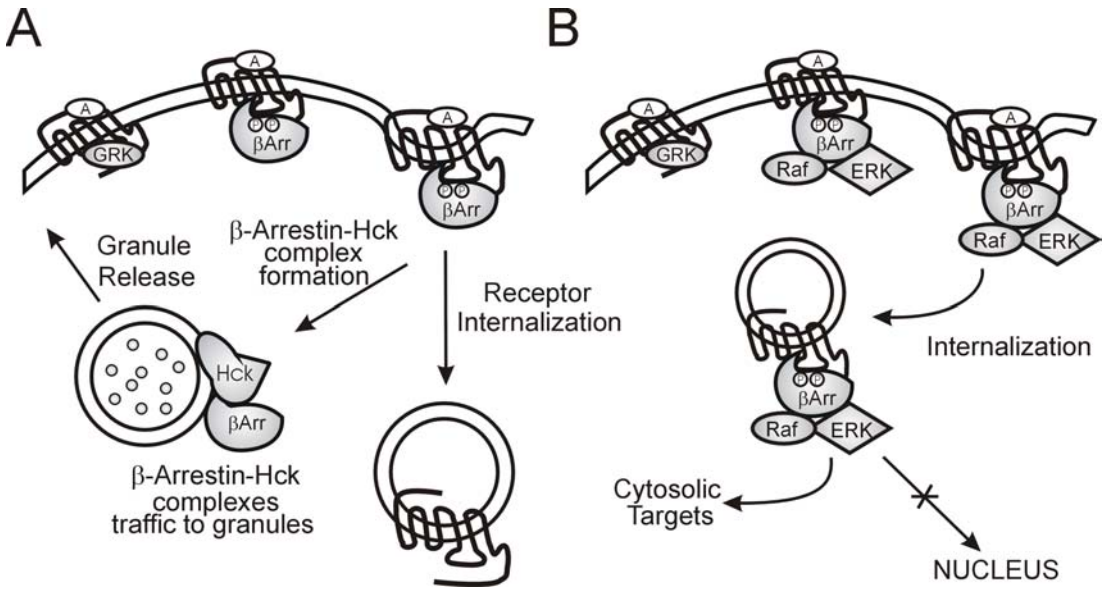


Figure 10. Schematic Diagram highlighting the targeting of tyrosine kinases to a GPCR via an interaction with β-arrestin followed by the either translocation of the complex to clathrin-coated pits or the dissociation of the complex from the receptor and its redistribution to neutrophillic granules.

Homers:

Family Members:

1. Homer1a – upregulated in response to seizures and LTP formation, acts as an endogenously expressed dominant-negative protein.

   Homer1b – other Homers are constitutively expressed

   Homer1c -proposed to couple receptors directly to IP3 receptor

2. Homer2 - interacts with cdc42
   - interacts with the cytoskeleton

3. Homer3 - attenuates constitutive activity

Homers are thought to couple metabotropic glutamate receptors 1/5 (mGluR) directly to the activation of IP3 receptor (channel) mediated increases in intracellular calcium concentrations. Homer1a/b/2/3 can homo- and hetero-dimerize with one another through a carboxyl-terminal coiled-coiled domain. Homer1a lacks this domain but retains the EVH (Ena Vasp Homology) domain that recognizes a PPXXFR motif the carboxyl-terminal tails of mGluR1 and 5 as well as in the IP3 receptor. Upregulation of Homer1a results in the displacement of constitutively expressed Homers from their binding sites thereby uncoupling the mGluRs and IP3 receptors from one another.

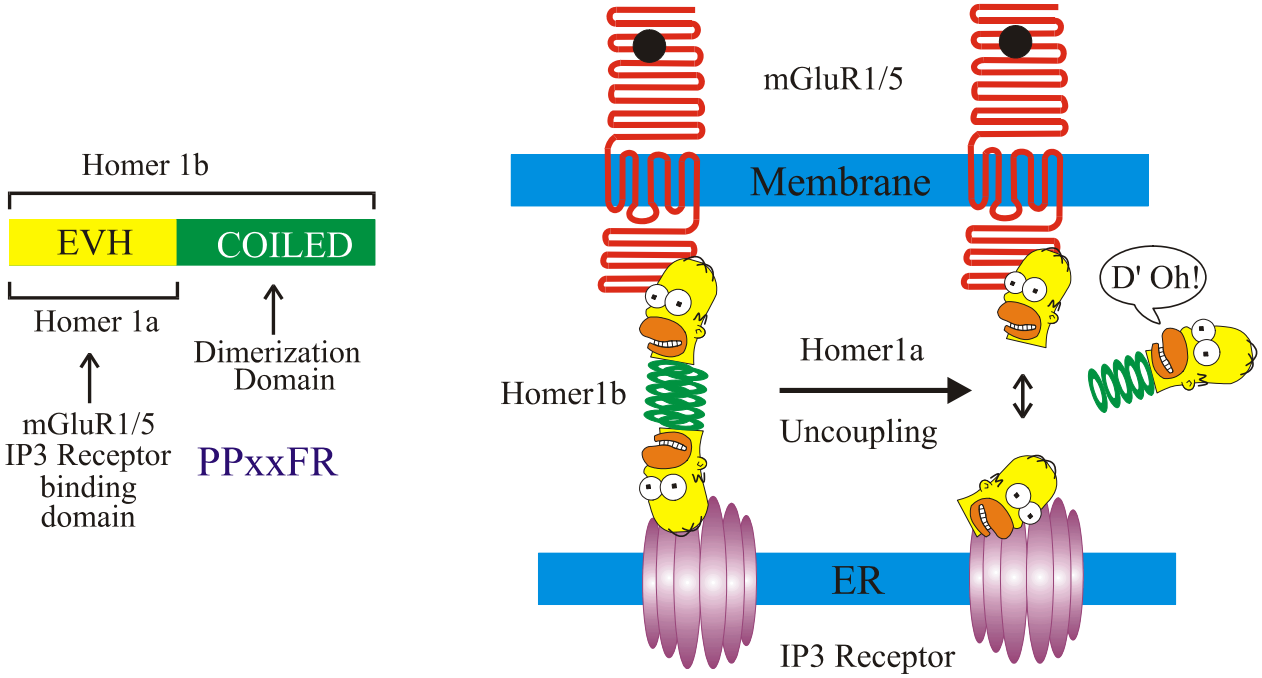


Figure 12. Schematic representation of Homer function.

Effect on Subcellular Localization

Homer1a
- allows mGluR localization to both axons and dendrites
- couples mGluRs to N-type calcium channels
- couples mGluRs to M-type potassium channels

Homer 1b
- promotes ER retention of mGluRs
- cooperates with SHANK to accumulate mGluRs at synapses

Homer 1c
- induces mGluR clustering at synapses

Homer 2
- targets mGluRs exclusively to dendrites