G-Proteins and GPCRs II

- Review of G-protein regulation
- GPCR dimerization and networking
- GPCR Pharmacology
- Ras proteins: soluble GTP-binding proteins
- Connections among pathways
- Note: Vision disobeys all the rules!
- Note 2: Need to add 15 minutes of case studies!

Sources: Gomperts, Voet and Voet
Science's STKE

Review Regulation of G-proteins

- When GDP is bound to G-proteins, they are inactive, when GTP is bound, they are active. Therefore the G-proteins have built-in molecular egg timers. G_{α} and $G_{\beta\gamma}$ subunits can have independent effects.
- In some cases, like EF-Tu, the egg timer enforces fidelity, in our current context it ensures that the signal lasts only a short time.
- GEFs potentiate guanine nucleotide exchange, typically GTP binding and hence activation. GPCRs are GEFs.
- GAPs and RGS proteins speed up the GTPase activity of the G-protein a subunit, hence inactivating it.
- GDI's like the $G_{\beta\gamma}$ subunits slow down GDP release, can activate or repress.
- Multiplicity of effects comes from multiplicity of GPCRs, G_{α}, G_{β} and G_{γ}.

GPCR Architecture

- Can be activated by light, biogenic amines, peptides, glycosylated hormones, Ca^{++}, or proteases

Table 3.5 Categories of 7TM receptors

Receptor properties	Ligands
Ligand binds in the core region of the 7 transmembrane helices	11-cis-Retinal (in rhodopsin) Acetylcholine Catecholamines Biogenic amines (histamine, serotonin, etc.) Nucleosides and nucleotides Leukotrienes, prostaglandins, prostacyclins, thromboxanes
Short peptide ligands bind partially in the core region and to the external loops	Peptide hormones (ACTH, glucagon, growth hormone, parathyroid hormone, calcitonin)
Ligands make several contacts with the N-terminal segment and the external loops	Hypothalamic glycoprotein releasing factors (TRH, GnRH)
Induce an extensive reorganization of an extended N-terminal segment	Metabotropic receptors for neurotransmitters (such as GABA and glutamate) Ca^{2+}-sensing receptors, for example on parathyroid cells, thyroidal (calcitonin secreting) C-cells and kidney juxtaglomerular apparatus
Proteinase-activated receptors	Receptors for thrombin and trypsin

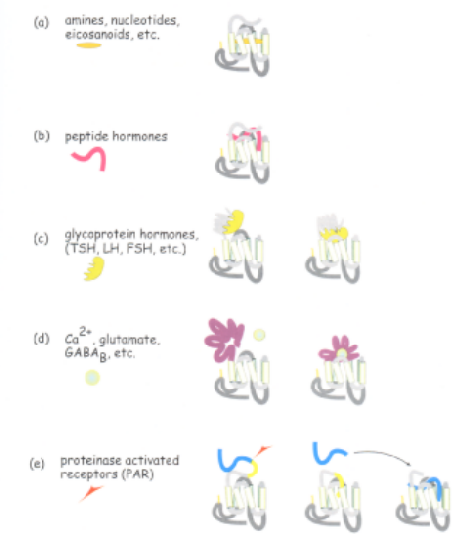


Figure 3.16 Ligand binding sites. The 7TM receptor is a jack of many trades, regulating a variety of different effectors and responding to ligands which come in many forms, having relative molecular masses in the range 32 (Ca^{2+}) to more than 10^4D. Most of the common low-mass hormones (such as adrenaline, acetylcholine) bind to sites within the hydrophobic core (a). Peptide and protein ligands are accommodated on the exterior face of the receptor (b, c). Although of low molecular mass, Ca^{2+} and the amino acids glutamate and GABA bind to extended N-terminal extensions, inducing new conformations which interact with the receptor (d). The proteinase-activated receptors are cleaved (e), the newly exposed N-terminus acting as an auto-ligand. The freed peptide may also interact separately with another receptor. Adapted from Ji et al.[34]

GPCR Pharmacology

- Structure-activity relationships (SARs) are obtained by making variations of lead compounds that inhibit the GPCR
- This is correlated with mutagenesis of the presumed binding pocket residues derived from homology to previous receptors
- As we have discussed, bacteriorhodopsin and rhodopsin aren't really much help

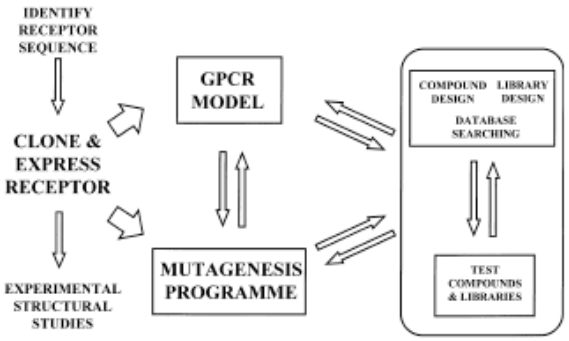
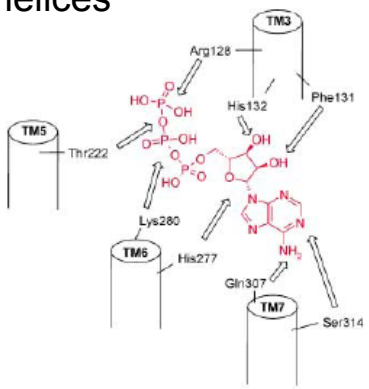


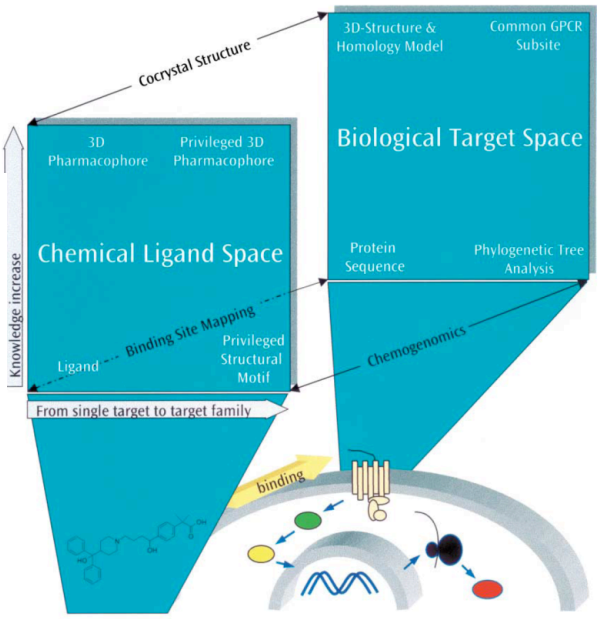
Fig. 8. GPCR modelling in drug design. Schematic diagram illustrating, as an idealised flowchart, how the receptor modelling and site-directed mutagenesis of GPCRs can be used synergistically to aid in the discovery and optimisation of novel chemical entities. Implicit in this a collaboration between modelling, medicinal chemistry, molecular biology, and pharmacology. The GPCR model informs construction of mutants, parameters for database searching, and the design of compounds and compound libraries.

Binding of Agonists/Antagonists

Typically small-molecule ligands contact several TM helices



Scheme 3. Schematic drawing of the interaction of ATP with the P2Y$_1$ receptor as derived from mutagenesis and modeling studies. For reasons of clarity the interaction with Arg310(TM7) is not shown. References are given within the text.

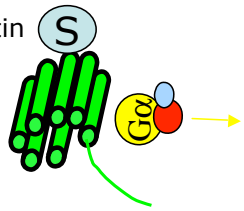


Knowledge for GPCR Lead Finding & Optimization

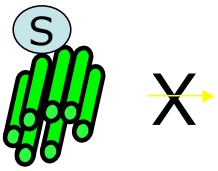
Receptor Dimerization

Functional D2R SSTR5 dimer

somatostatin + SSTR5 R

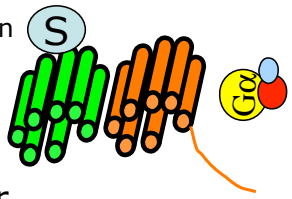


somatostatin + SSTR5 del C



X

somatostatin + SSTR5 del C + D2 receptor



Recovery of SS Signaling via D2R

From STKE

Receptor Networks

- Receptor mosacis can include both homotypic and heterotypic interactions to integrate output.
- Positive cooperativity allows for a sensitive response to weak signals
- Negative cooperativity allows for protection against elevated agonist levels and a response over a wide range of concentration.
- See Agnati et al., (2005) TiBS 30, 188.
- The “signalosome”
- Does this remind you of anything?

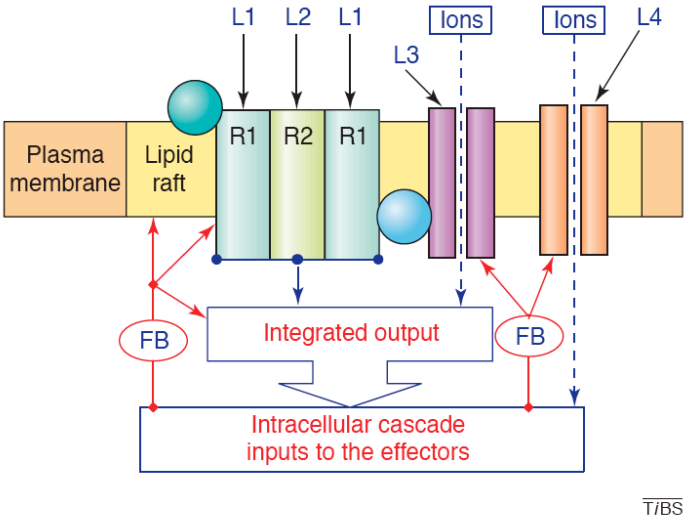


Figure I. Schematic representation of a horizontal molecular network localized at the plasma-membrane level. The interacting receptors form a RM that works as an integrated recognition-transducing system of some of the ligands (chemical messengers) that impinge on the cell membrane. Abbreviations: FB, feedback signal; L, ligand; R, receptor; R1and R2, receptor mosaics.

Ras Proteins

- Prototype of soluble G-proteins
- Mutations that lead to persistent GTP binding are oncogenic
- Activates MAP kinase pathway, which leads to transcription of genes required for proliferation
- Homologues in yeast, fly are very similar
- Localized to plasma membrane by farnesylation

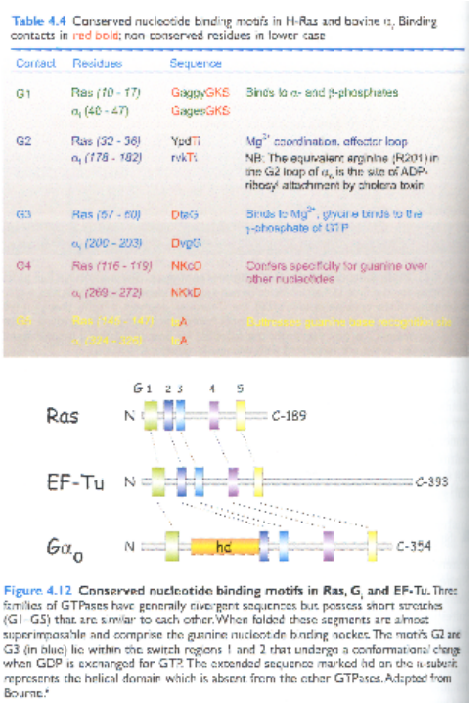
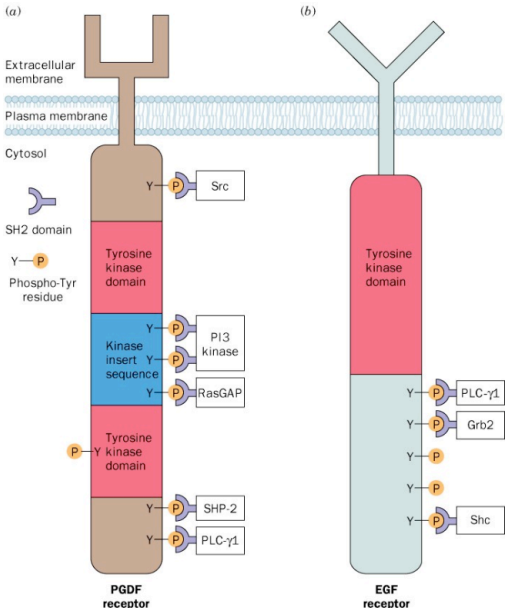
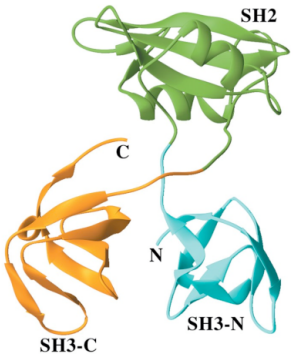


Figure 4.12 Conserved nucleotide binding motifs in Ras, G_i and EF-Tu. Three families of GTPases have generally divergent sequences but possess short stretches (G1–G5) that are similar to each other. When folded these segments are almost superimposable and comprise the guanine nucleotide binding pocket. The motifs G2 and G3 (in blue) lie within the switch regions 1 and 2 that undergo a conformational change when GDP is exchanged for GTP. The extended sequence marked hd on the α-subunit represents the helical domain which is absent from the other GTPases. Adapted from Bourne.*

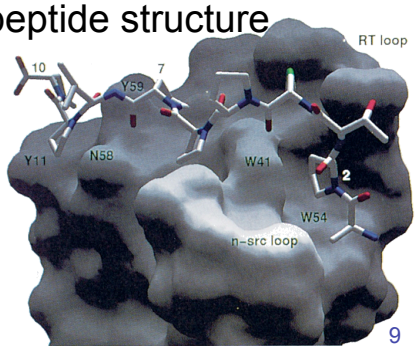
Ras Recruitment





Grb2

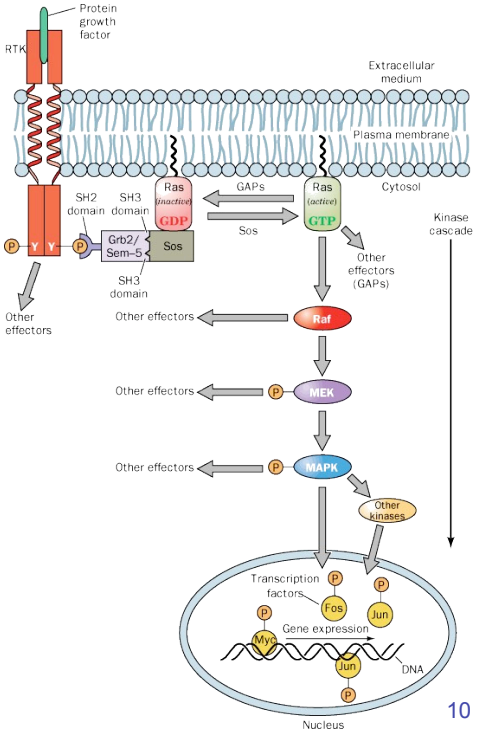
SH3-peptide structure



V+V 3/e

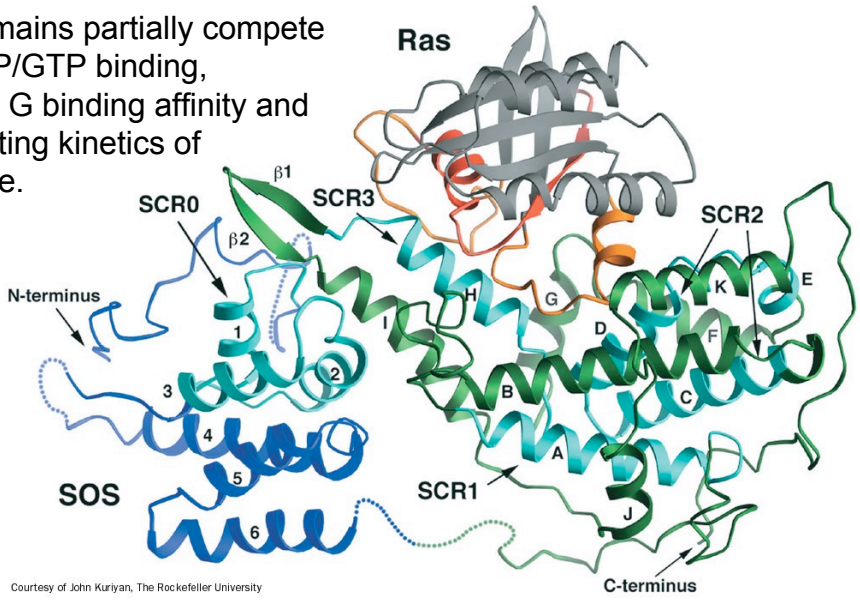
Ras Mediation of RTK Signalling

- The Grb2 adaptor protein has SH2 domains that recruit it to phosphorylated tyrosine residues on RTKs, including the EGF receptor.
- The insulin receptor recruits Grb2 indirectly: the phosphorylated InsR recruits Shc through Shc's SH2 domain and then Tyr-phosphorylates Shc to make it a ligand for the SH2 domain of Grb2.
- Grb2 has two SH3 domains, which bind proline-rich binding motifs on the mSos protein (mammalian homolog of the Drosophila Son of Sevenless adaptor, where Sevenless is an RTK that regulates development of the R7 photoreceptor cell). So Sos is recruited to the plasma membrane (Sos and Grb2 are stably associated).
- mSos is the GEF for Ras, so Ras is recruited to the membrane and activated
- In contrast, the platelet-derived growth factor PDGF recruits RasGAP, which activates the GTPase activity of Ras. Common oncogenic Ras mutants are insensitive to RasGAP, hence always on.
- Ras activates the MAP kinase proliferation pathway through interaction with the effector Raf, a Ser/Thr kinase.



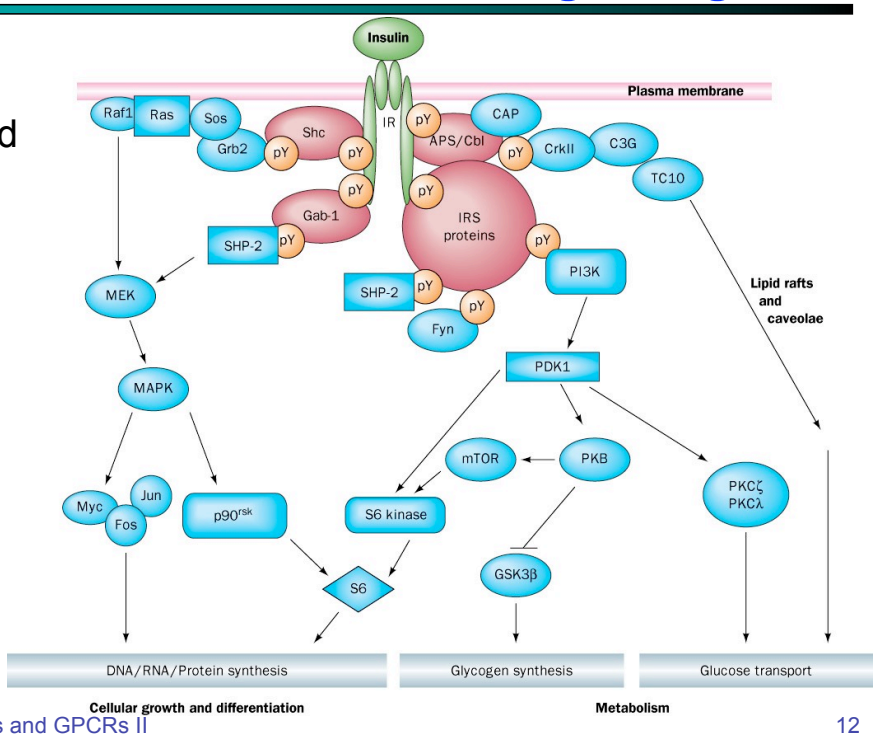
Guanine Nucleotide Exchange

- Structure of Ras/Sos complex
- SCR domains partially compete with GDP/GTP binding, reducing G binding affinity and accelerating kinetics of exchange.



The Left Hand of Insulin Signalling

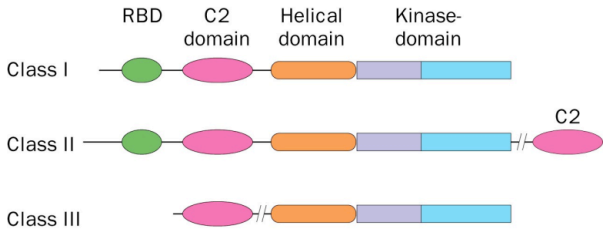
Insulin signals fed state, hence enables growth.

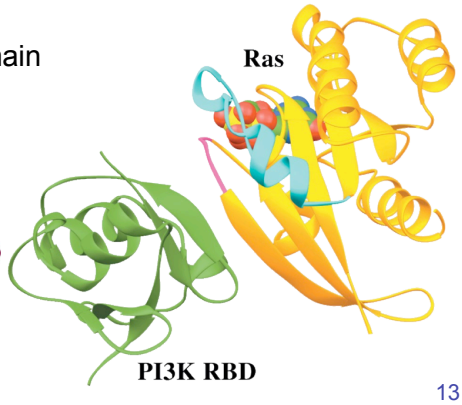


PI3K is an effector for Ras

- Recall that PI3K (phosphoinositide 3-kinase) is recruited to phosphorylated IRS1 via its regulatory p85 subunit, which eventually leads to PKB/Akt activation, inactivation of glycogen synthase kinase 3β, dephosphorylation of glycogen synthase by PP1, and glycogen synthesis.
- Recall further that Akt phosphorylates Bad, therefore decreasing its proapoptotic activity.
- The p110 catalytic subunit of PI3K is activated by binding Ras•GTP
- So…Ras activation of PI3K leads to phosphorylation of Bad.
- Why does this make sense? Ras simultaneously signals proliferation through the MAPK pathway and also inhibits apoptosis through PI3K. Throw both switches.

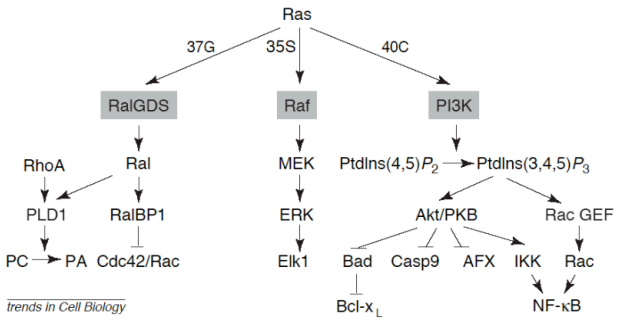
PI3K p110 isoforms: RBD = Ras binding domain





Ras Summary

- Shields et al., Trends Cell Biol. 2000.
- The RalGDS connection means that Ras can activate the GEFs that then activate other soluble G-proteins.



BOX 1 – CANDIDATE EFFECTORS OF RAS

Mammalian

- Serine/threonine kinases: c-Raf-1, A-Raf, B-Raf, MEKK1, PKC-ζ
- Ras GTPase-activating proteins (GAP): p120 GAP , neurofibromin (NF1)
- Phosphoinositide 3-kinase (PI3K) lipid kinases: p110α, p110β, p110γ, p110δ
- Ral GDP–GTP exchange factors (GEF): RalGDS, RGL/Rsb2, RGL2/Rlf
- Rin1 Abl-interacting protein
- Nore1 novel protein
- AF-6 fusion partner in chromosomal translocations associated with human acute leukaemias

Other

- Adenylyl cyclase (*Saccharomyces cerevisiae*)
- Scd1 Cdc42 GEF (*Schizosaccharomyces pombe*)
- Byr2 serine/threonine kinase (*S. pombe*)
- Lin-45 serine/threonine kinase (*Caenorhabditis elegans*)
- PLC210 phospholipase C (*C. elegans*)
- D-Raf serine/threonine kinase (*Drosophila melanogaster*)

Network Architecture

- Junctions split incoming signals to several output channels (RTKs, Ras)
- Nodes integrate several inputs to give a common output (Adenylate cyclase)
- Allows for propagation of a signal throughout a network or dissipation of the signal
- Buzzphrase: “Emergent properties of networks”
- Jordan et al., Cell 2000.
- Will require huge amounts of data and mathematical modeling to understand how this works.

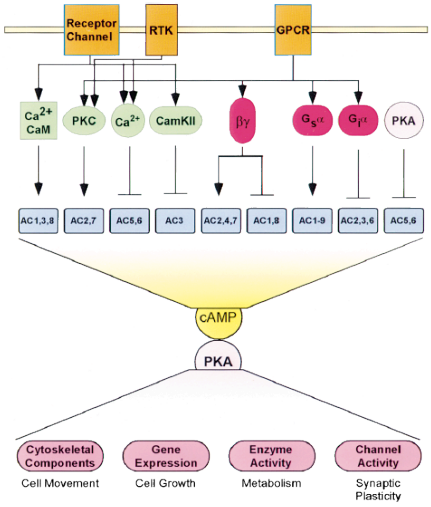
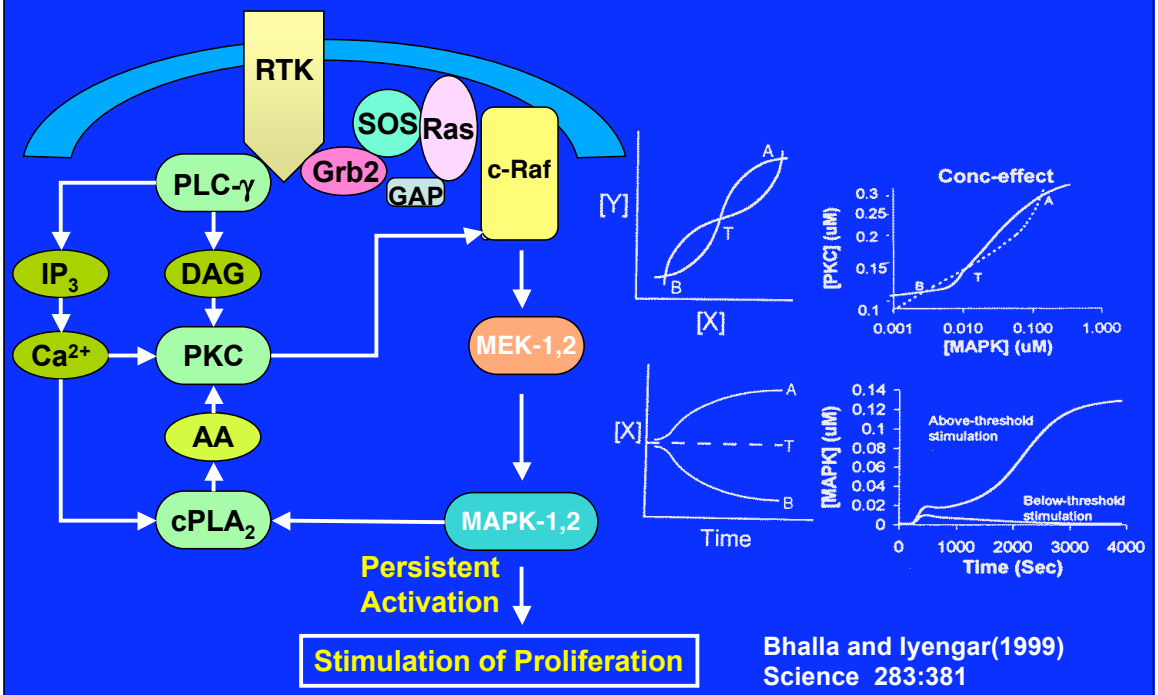


Figure 1. Adenylyl Cyclases as Examples of a Junction

The signal receiving capabilities of the various adenylyl cyclase isoforms and the capability of the cAMP-dependent protein kinase (PKA) to regulate various physiological functions are shown. Receptor channel, ligand gated channel (e.g., NMDA receptor); RTK, receptor tyrosine kinase; GPCR, G protein–coupled receptor. Stimulatory signals are shown as arrows and inhibitory signals as plungers. The various cellular components or processes regulated by PKA are shown in the red ovals and the resultant physiological functions are given below.

Consequence of Networking: A feedback loop that displays bistability



Bhalla and Iyengar(1999) Science 283:381

Effector Dimerization

- Nine isoforms talk to different G proteins, Ca++, PKA, PKC
- Adenylate cyclases are dimers (at least)
- Dimerization allows for integration of signals from different heterotrimeric G proteins, through interaction with either α or $\beta\gamma$ subunits.
- A molecular “coincidence detector?” (How do scintillation counters work?)

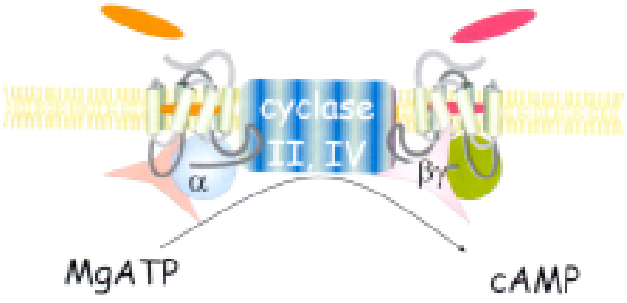
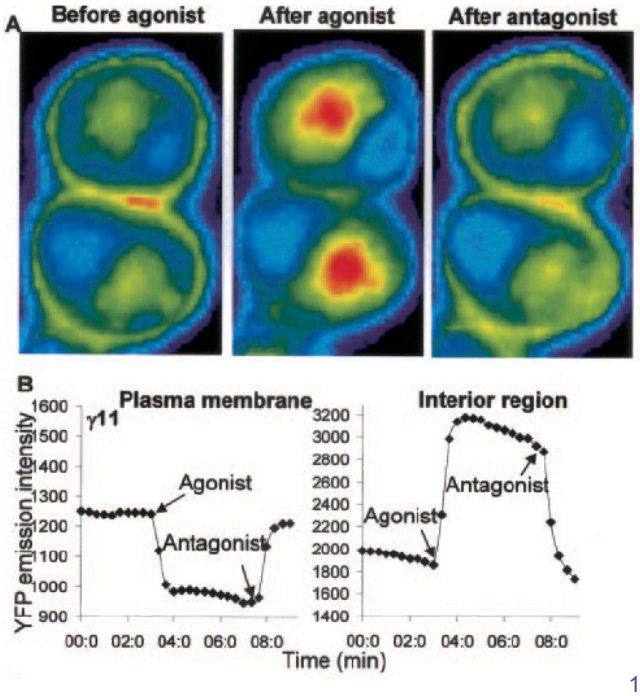


Figure 5.4 Adenylyl cyclase as a coincidence detector, integrating the signals from two G-proteins.

Receptor Localization

- Phosphorylated receptors recycle via endocytosis of clathrin-coated vesicles followed by dephosphorylation in the endosome and return to the plasma membrane.
- Heterotrimeric G proteins were observed to relocate to the Golgi apparatus (involved in protein trafficking) after activation, then recycle to the plasma membrane after inactivation. Mechanism entirely unclear. (Akgoz et al., JBC 2004)
- Proposed to offer a mechanism for rapid desensitization, mediated by γ subunit.



Summary

- Networks of GTP-binding protein signalling are activated by extracellular signals through GPCRs and RTKs, via adaptors, GEF activities.
- Many and varied downstream effectors amplify, split, and integrate signals
- Signals are attenuated by GAP activities, network architecture, receptor phosphorylation, and receptor and G-protein trafficking