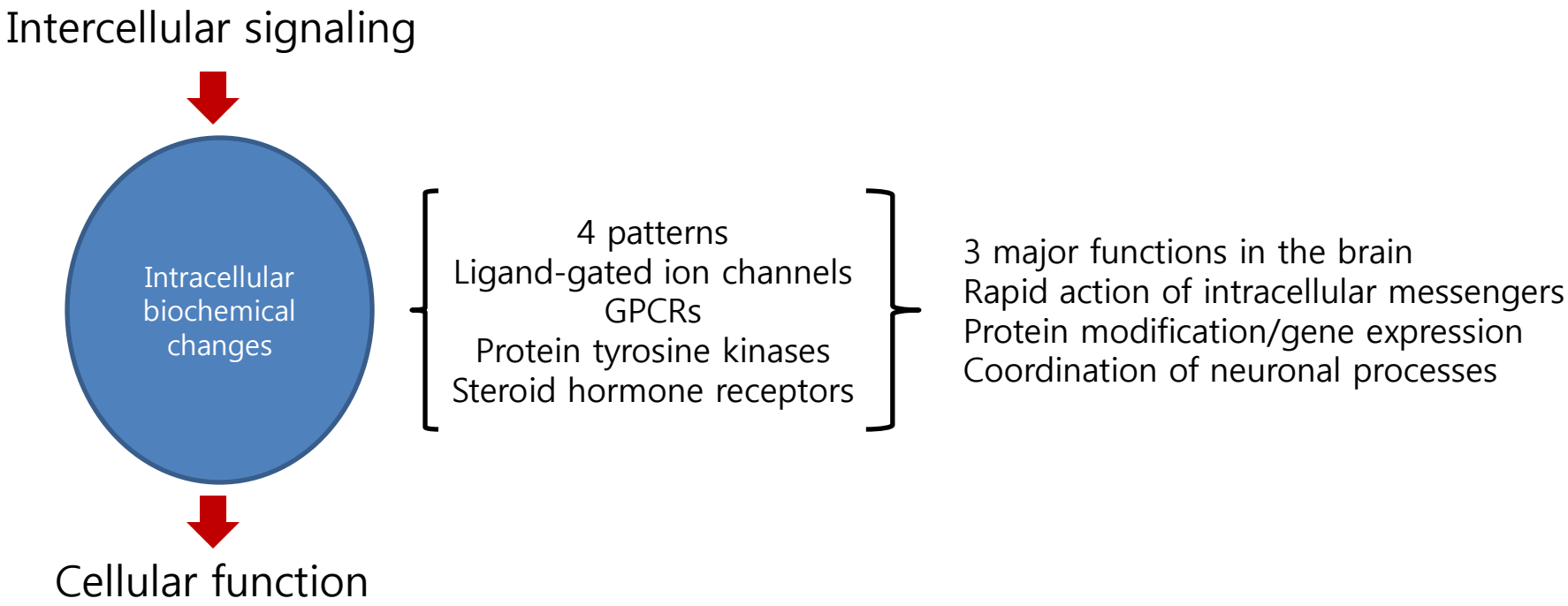
Chap 5. Signal transduction pathways in the brain



Ligand-gated ion channels

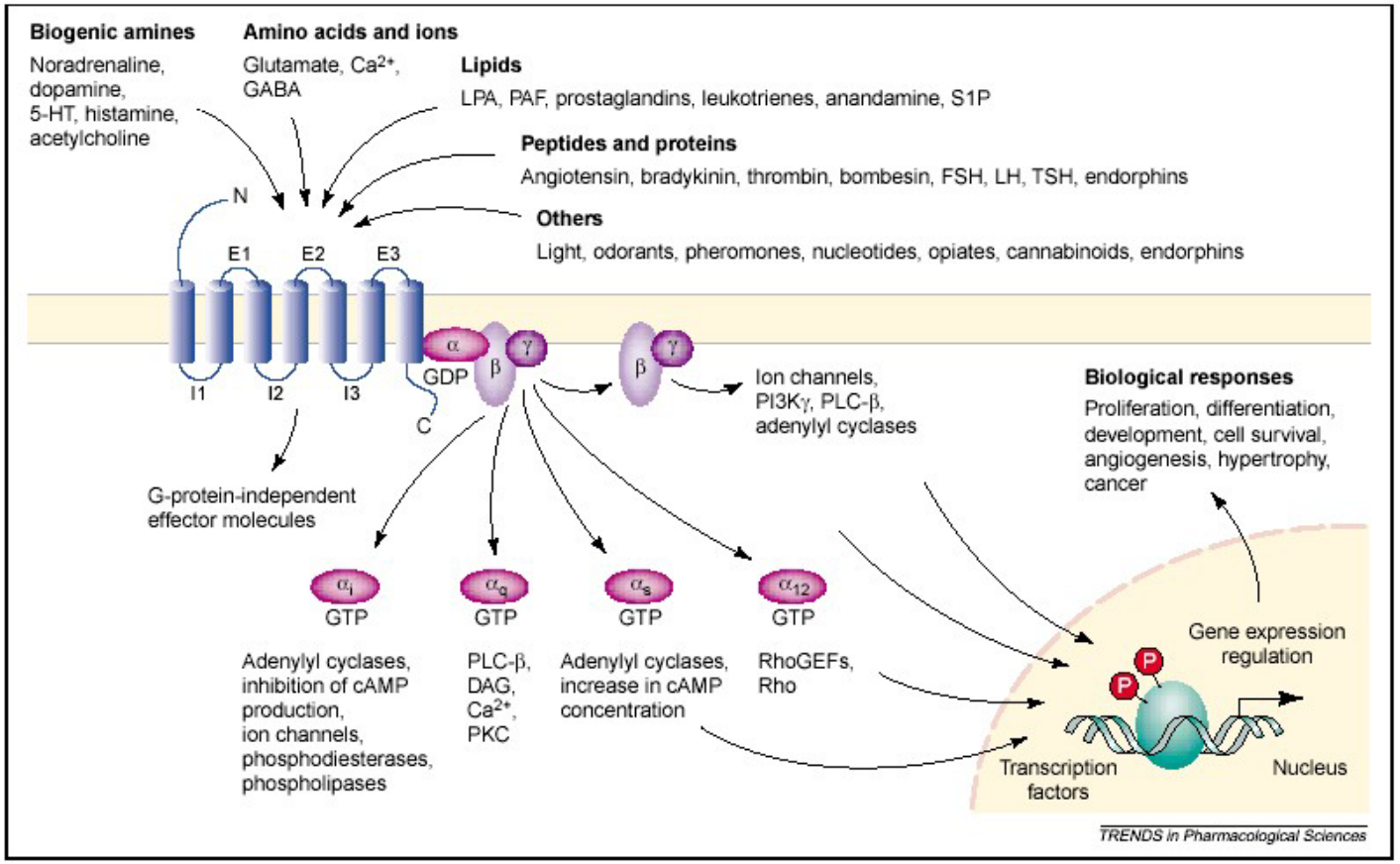
GPCRs: heterotrimeric G proteins (α,β,γ)
small GTPases
2nd messengers & protein phosphorylation

Protein tyrosine kinases:
Receptor tyrosine kinases (RTKs): 20 different classes
nonreceptor tyrosine kinases (cytoplasmic)

Steroid hormone receptors:
cytoplasmic receptors as transcription factors

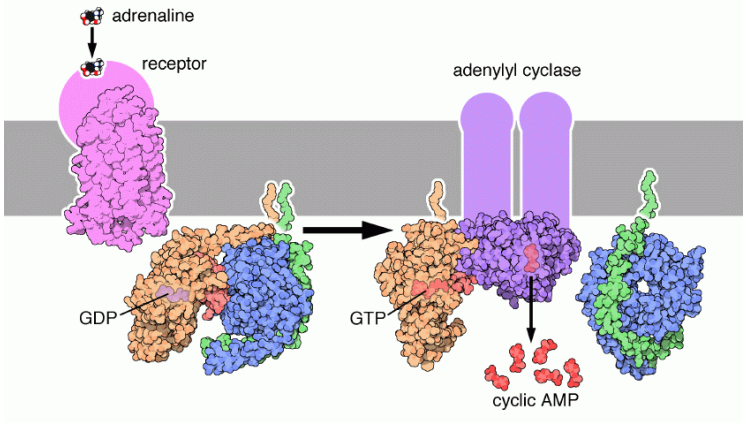
G proteins and second messengers

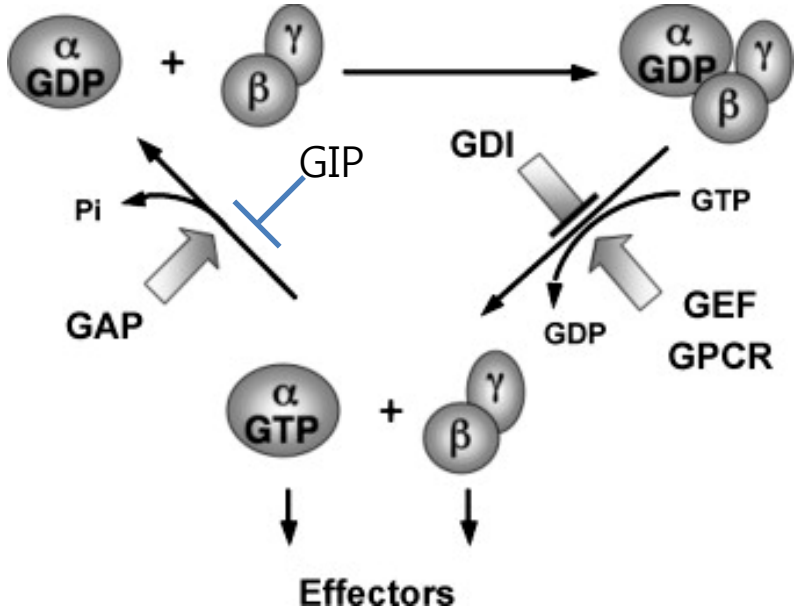
G proteins
Gs family
Gi family
Gq family
Gt family
Gg family
G12 family
Small-MW G proteins (small GTPases)



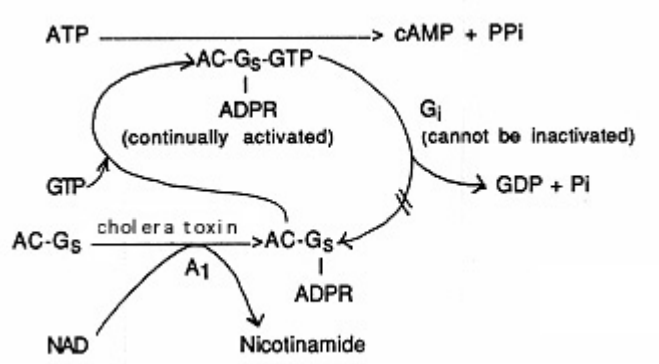
http://www.ibibiobase.com/projects/db-drd4/G_protein.htm

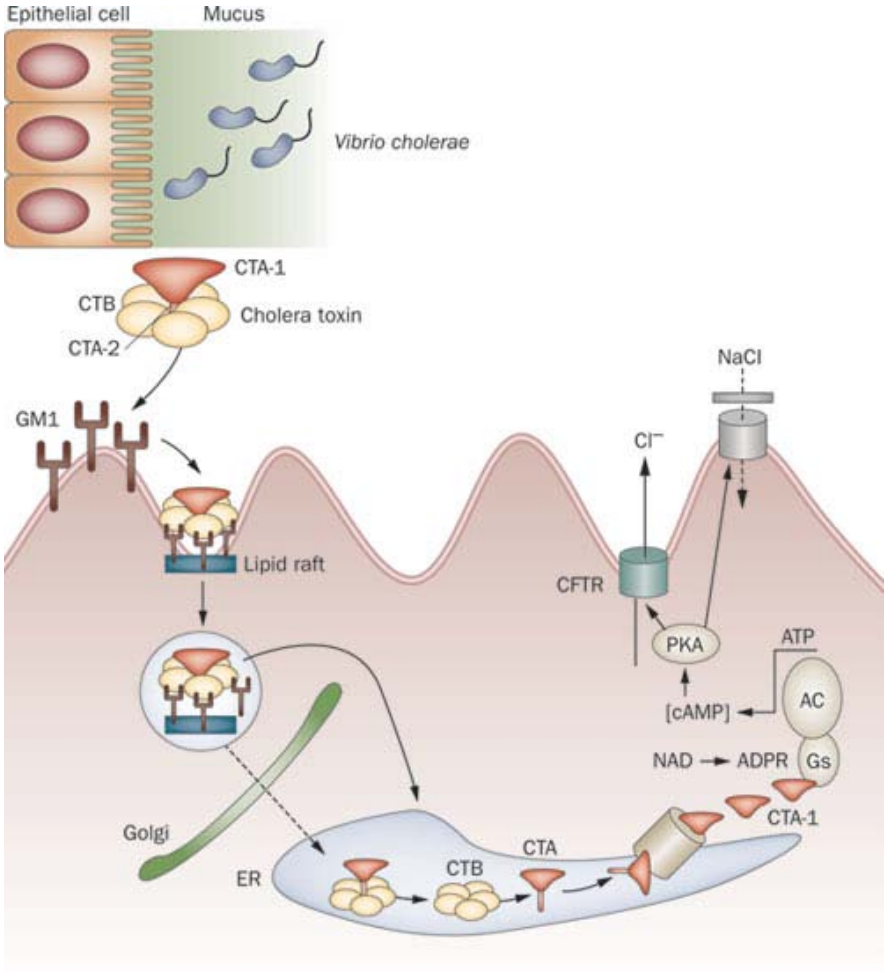
RGS: regulator of G-protein signaling" (RGS)
GAP: GTPase-accelerating protein
GIP: GTPase-inhibiting proteins
GDI: guanine nucleotide dissociation inhibitor (GDI)
GEF: guanine nucleotide exchange factor



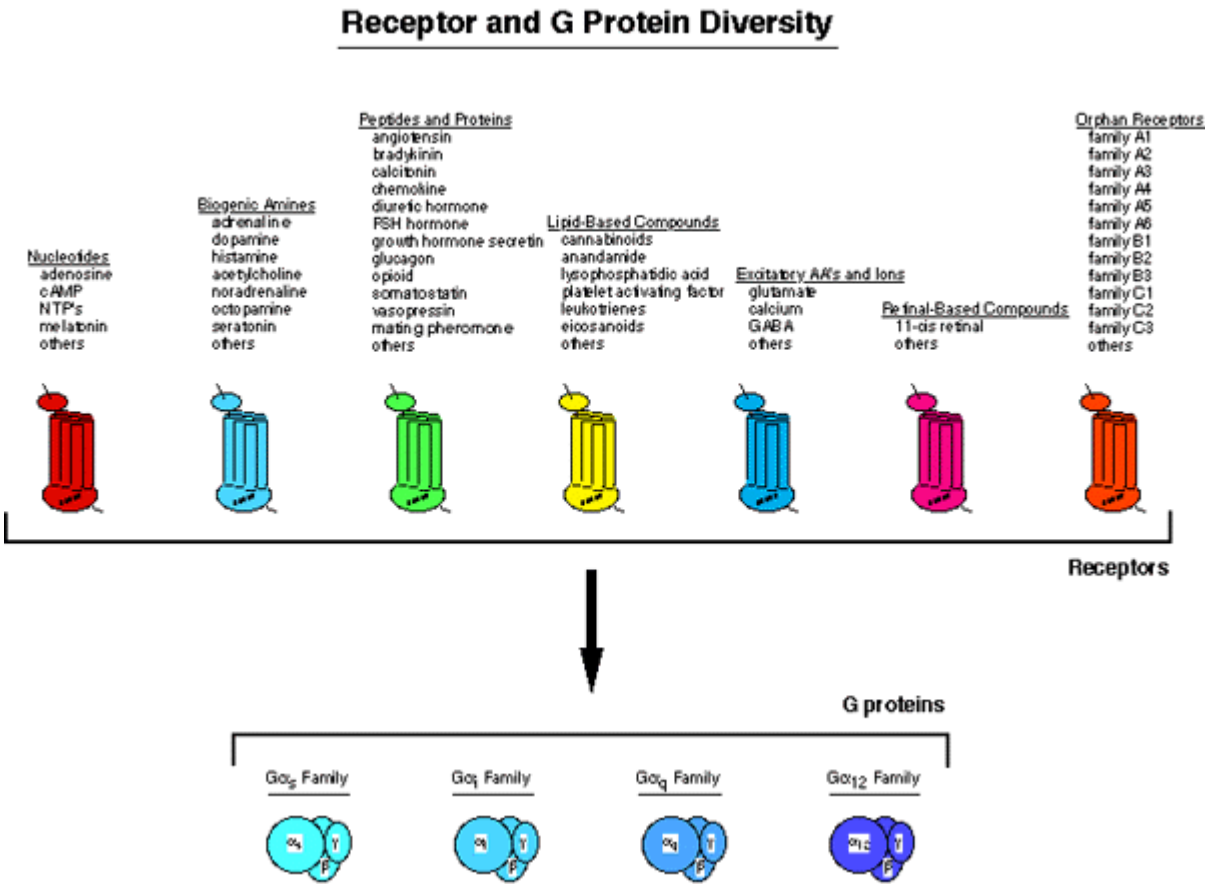


Cholera toxin: ADP-ribosylates Gαs
Pertussis toxin: ADP-ribosylates Gαi
Diphtheria toxin: ADP-ribosylates small G protein





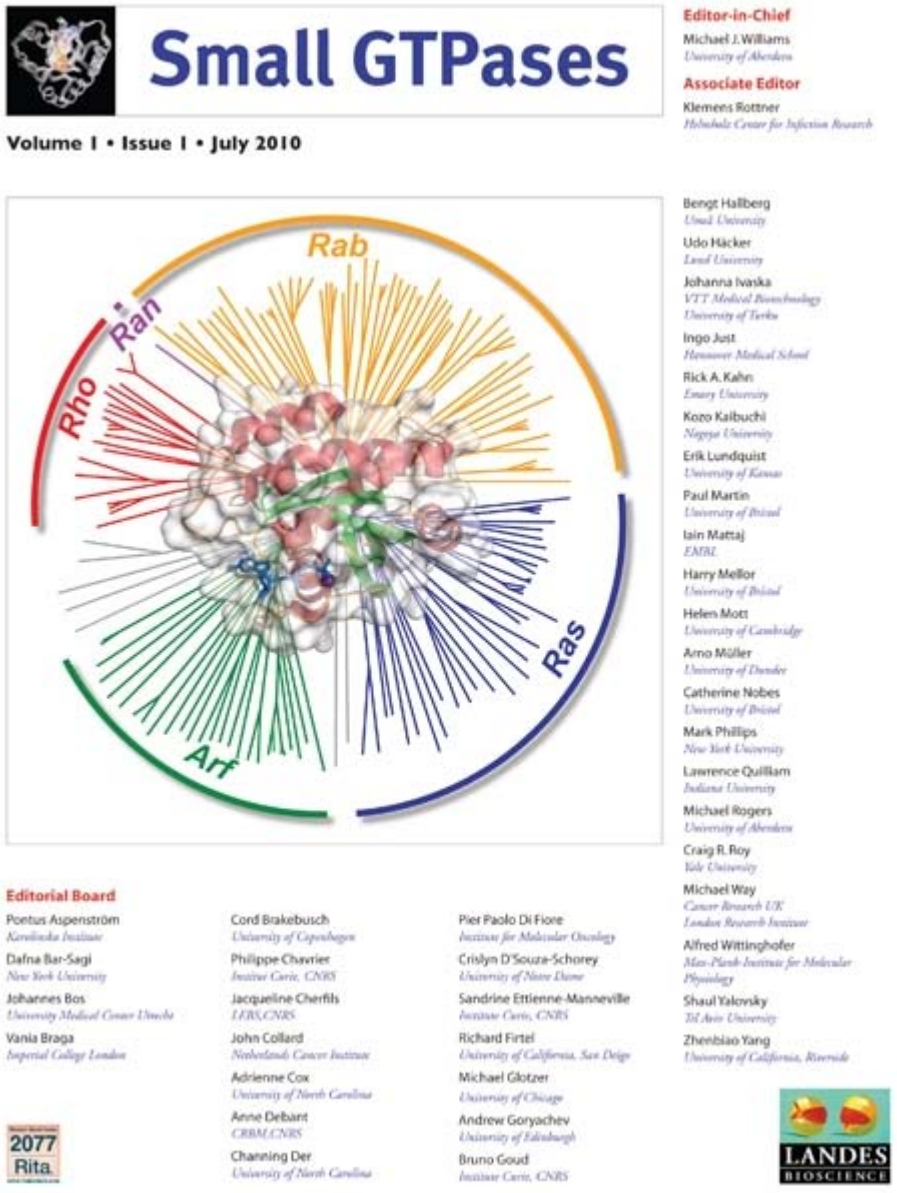
After ingestion, V. cholerae colonizes the small intestine and secretes cholera toxin, which has a doughnut-like structure with a central enzymatic toxic-active A (A_1+A_2) subunit associated with pentameric B subunits (B5). After binding to GM1 ganglioside receptors, mainly localized in lipid rafts on the cell surface, the toxin is endocytosed and travels to the ER via a retrograde pathway which—dependent on cell type—may or may not involve passage through the Golgi. In the ER, the A subunit dissociates from the B subunits and through translocation via the ER degradasome pathway, A1 can reach the cytosol where it can rapidly refold. It binds to and ADP-ribosylates Gs, stimulating the AC complex to produce increased cellular levels of cAMP, leading to activation of PKA, phosphorylation of the major chloride channel, CFTR, and secretion of chloride (Cl^-) and water. Cholera toxin-induced chloride (and bicarbonate) secretion is especially pronounced from intestinal crypt cells, whilst in villus cells the increased cAMP levels instead mainly inhibits the normal uptake of NaCl and water.[14] Abbreviations: AC, adenylate cyclase; ADPR, ADP ribose; cAMP, cyclic AMP; CTA, cholera toxin A; CTB, cholera toxin B; CTFR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; Gs, GTP- binding protein, Gs; PKA, protein kinase A. Nature Reviews Gastroenterology & Hepatology 8, 701-710 (December 2011)



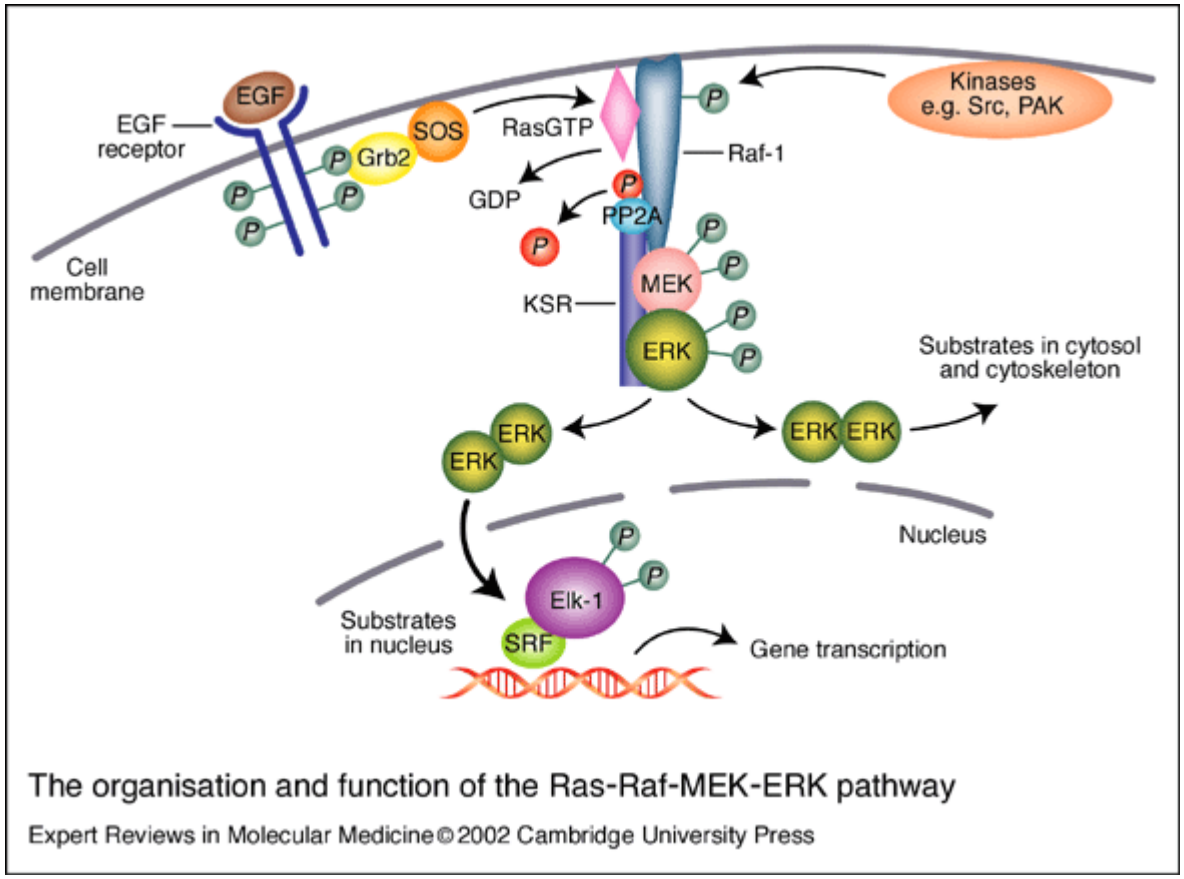
Classes of G protein-coupled receptors (GPCR) and the G prot eins they regulate. http://spot.colorado.edu/~falke/figures/fig10/figure10.html

Small GTP-binding proteins (also known as small GTPases) are essential for multiple cellular processes such as
cell proliferation (Ras),
dynamics of the cytoskeleton (Rho and Rop),
membrane trafficking (Arf and Rab) and
nucleo-cytoplasmic transport (Ran).

They are known to be involved in numerous physiological processes including embryogenesis, establishment and/or maintenance of polarity, adhesion, migration and differentiation of numerous cell types.
Small GTPases 1:1, 1-1; July/August 2010; © 2010 Landes Bioscience

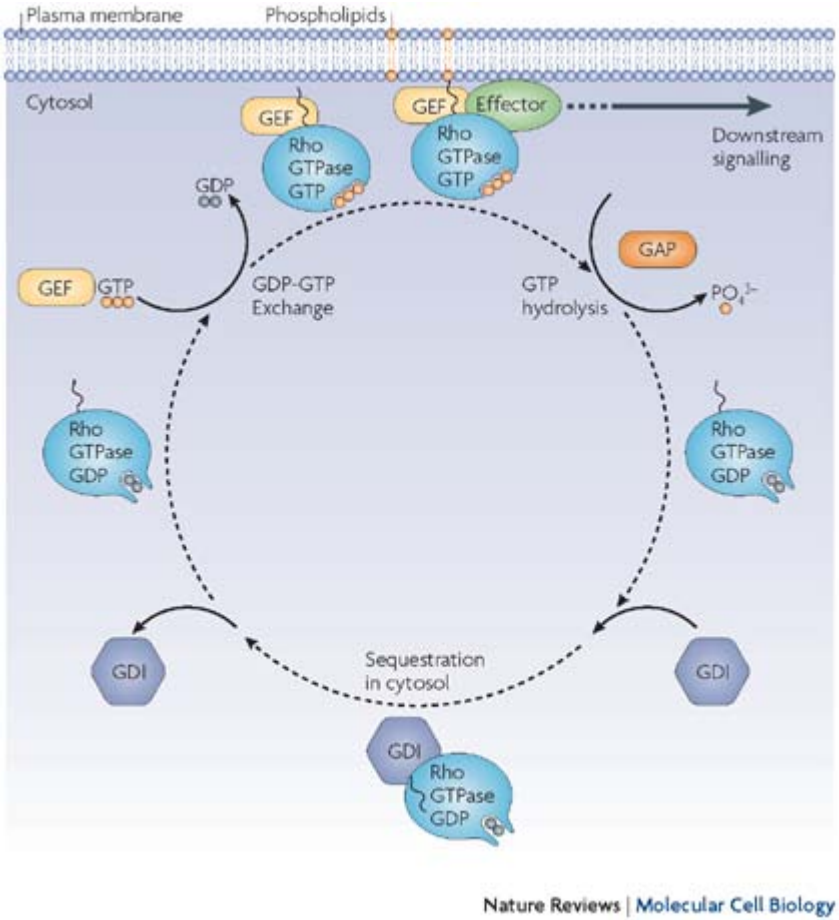


Small GTPases
Ras Pathway

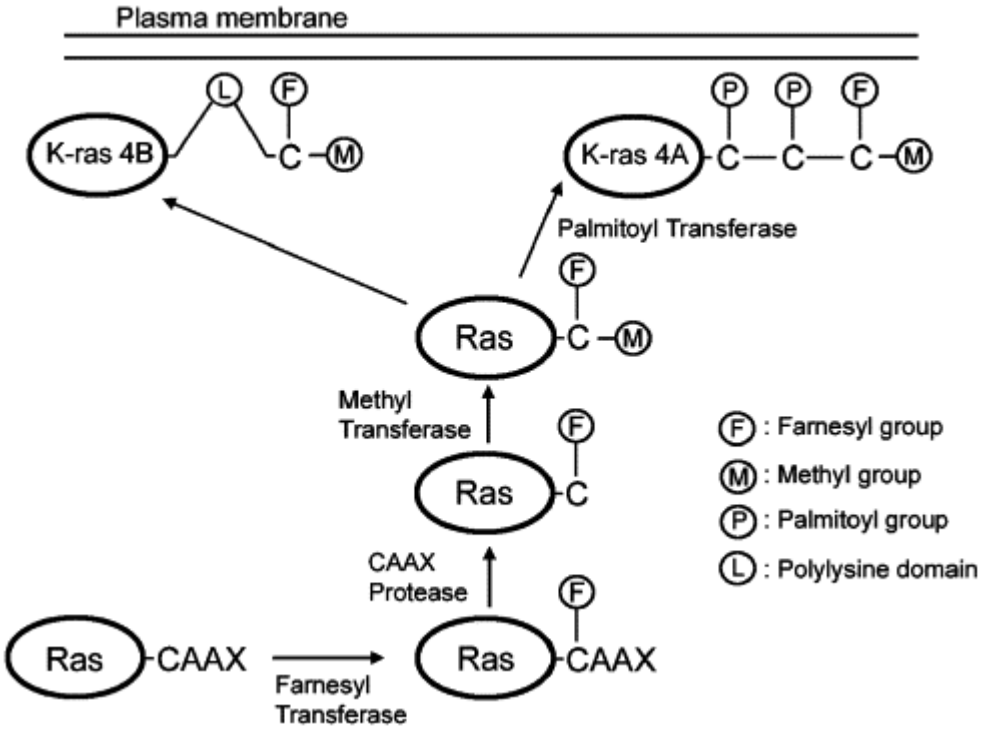


The binding of epidermal growth factor (EGF) induces receptor dimerisation and autophosphorylation (P) on tyrosine residues. These phosphotyrosines function as docking sites for signalling molecules including the Grb2–SOS complex, which activates the small G-protein Ras by stimulating the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP). This exchange elicits a conformational change in Ras, enabling it to bind to Raf-1 and recruit it from the cytosol to the cell membrane, where Raf-1 activation takes place. Raf-1 activation is a multi-step process that involves the dephosphorylation of inhibitory sites by protein phosphatase 2A (PP2A) as well as the phosphorylation of activating sites by PAK (p21$^{rac/cdc42}$-activated kinase), Src-family and yet unknown kinases. Activated Raf-1 phosphorylates and activates MEK (MAPK/ERK kinase), which in turn phosphorylates and activates extracellular-signal-regulated kinase (ERK). The interaction between Raf-1 and MEK can be disrupted by RKIP (Raf kinase inhibitor protein; not shown). The whole three-tiered kinase cascade is scaffolded by KSR (kinase suppressor of Ras). Activated ERK has many substrates in the cytosol [e.g. cytoskeletal proteins, phospholipase A2, and signalling proteins including tyrosine kinase receptors, oestrogen receptors, SOS, signal transducer and activator of transcription proteins (STATs) and others (Refs 10, 11)]. ERK can also enter the nucleus to control gene expression by phosphorylating transcription factors such as Elk-1 and other Ets-family proteins. Grb2, growth-factor-receptor-binding protein 2; SOS, 'son of sevenless'; SRF, serum response factor (animated version of Fig. 2) **(fig002wkg)**.

Rho family GTPases and their regulators

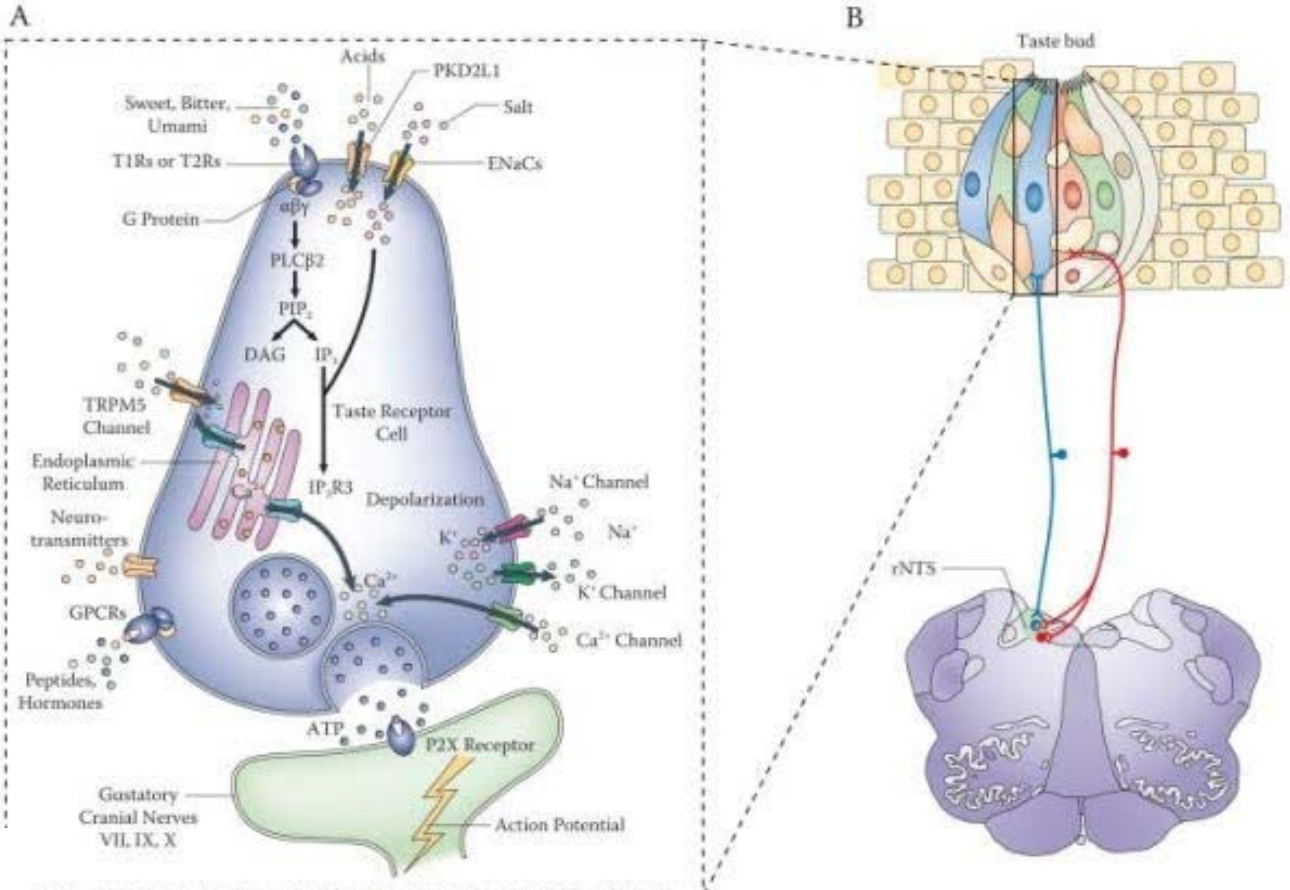


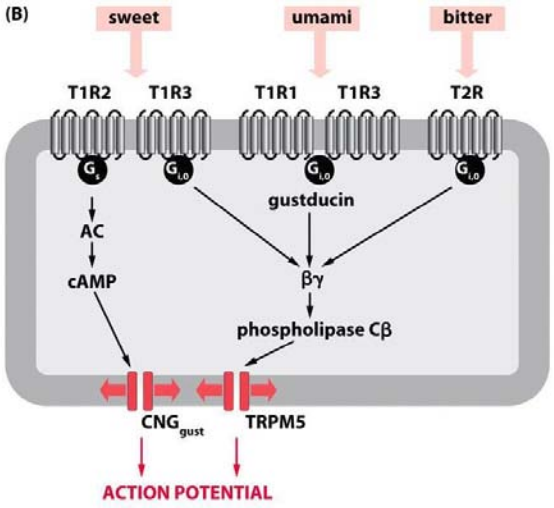
Rho family GTPases function as molecular switches and cycle between an active, GTP-bound state that is predominantly associated with membranes, and an inactive, GDP-bound state that is present in the cytoplasm. GTP binding induces a conformational change in Rho GTPases, thereby promoting their interaction with many effector molecules that control downstream signalling pathways. The temporal and spatial regulation of GTPase activity is an important element in the control of Rho GTPase signalling and takes place at three levels: GDP–GTP exchange, GTP hydrolysis and inhibition of GDP dissociation Nature Reviews Molecular Cell Biology 9, 846-859 (2008)



Post-translational modification of Ras. All Ras proteins contain the CAAX motif at the carboxy terminus, directing farnesylation on the cysteine residue by farnesyltransferase. Some Ras proteins are also substrates for geranylgeranyltransferase, particularly in the presence of farnesyl transferase inhibitors. Next, the terminal AAX tetrapeptide is removed by the CAAX protease RceI, followed by methylation of the terminal cysteine residue. K-ras proteins are differentially modified at this point, with K-ras 4A receiving palmitoyl on cysteine residues near the carboxy terminus, while K-ras 4B utilizes a polylysine domain for efficient membrane localization. BBA (2005) 1756,127-144.

Taste receptors
(G_g: gustducin)

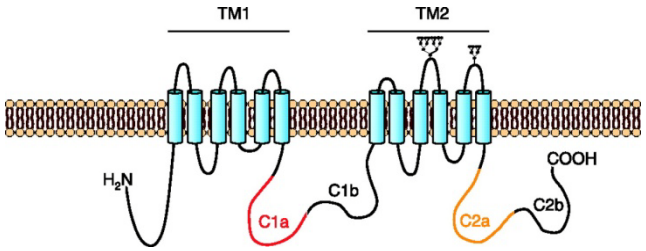


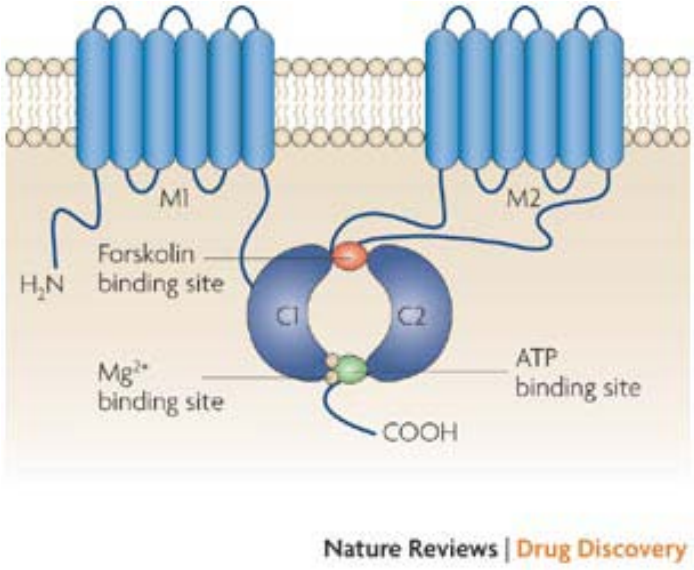


nce 2009)

Second messengers

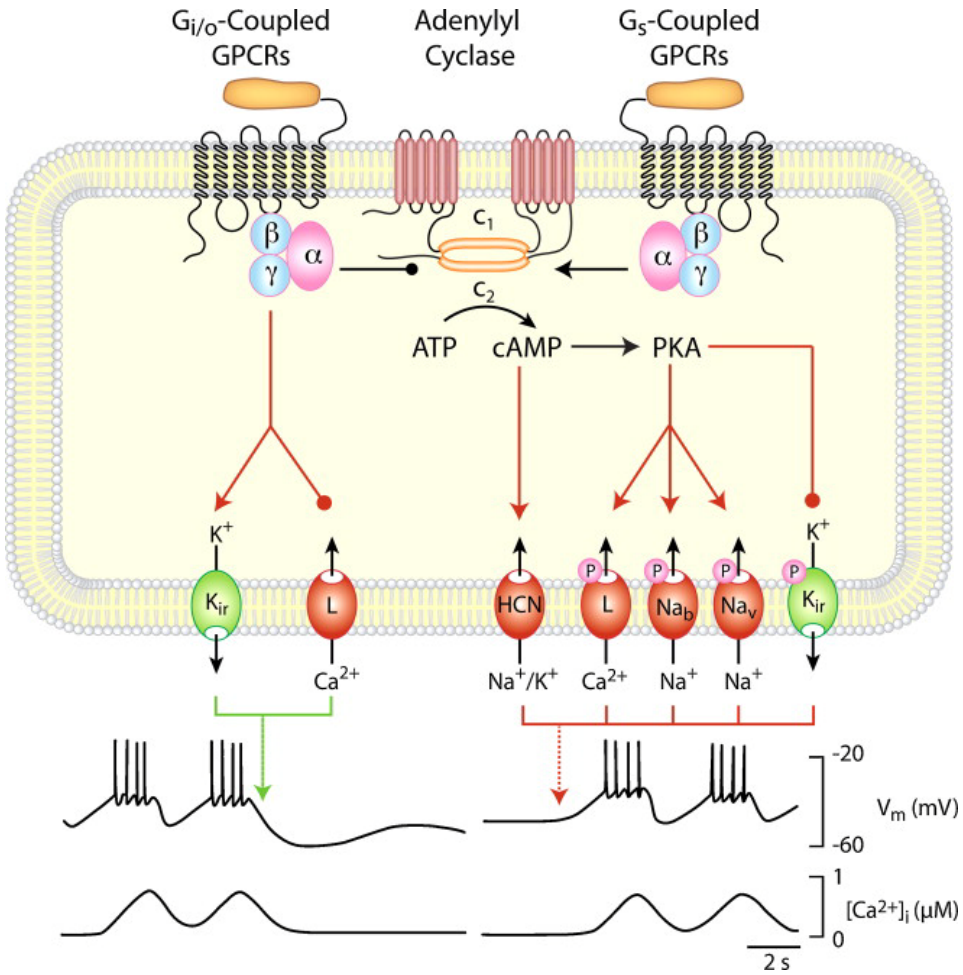
Adenylyl cyclases





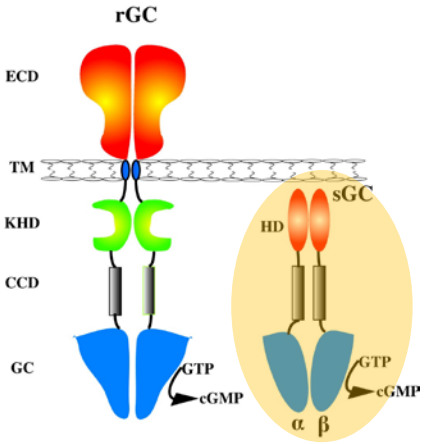
All adenylyl cyclases (ACs) consist of a short and variable amino-terminus, followed by two repeats of a module composed of six transmembrane spans (M1 and M2) and two cytoplasmic domains of approximately 40 kDa each (C1 and C2) as shown in the figure below. The two cytosolic domains form the catalytic active site. The tertiary structures of both adenylyl cyclase domains are nearly identical and together they form a pseudosymmetrical enzyme. The catalytic active site is created at the interface of C1 and C2 by residues contributed from both domains. As the active site is shared between the two domains, association of the two catalytic domains in the proper orientation is required for catalytic activity. Gs binds to a crevice on the outside of C2 and to the N-terminal portion of C1 and rearranges both domains, as does forskolin, by inducing a conformational change. By contrast, Gi binding to the C1 domain interferes with this conformational change and by inhibits enzymatic activity. Nature Reviews Drug Discovery 8, 321-335 (April 2009)

G protein regulation of ion channels

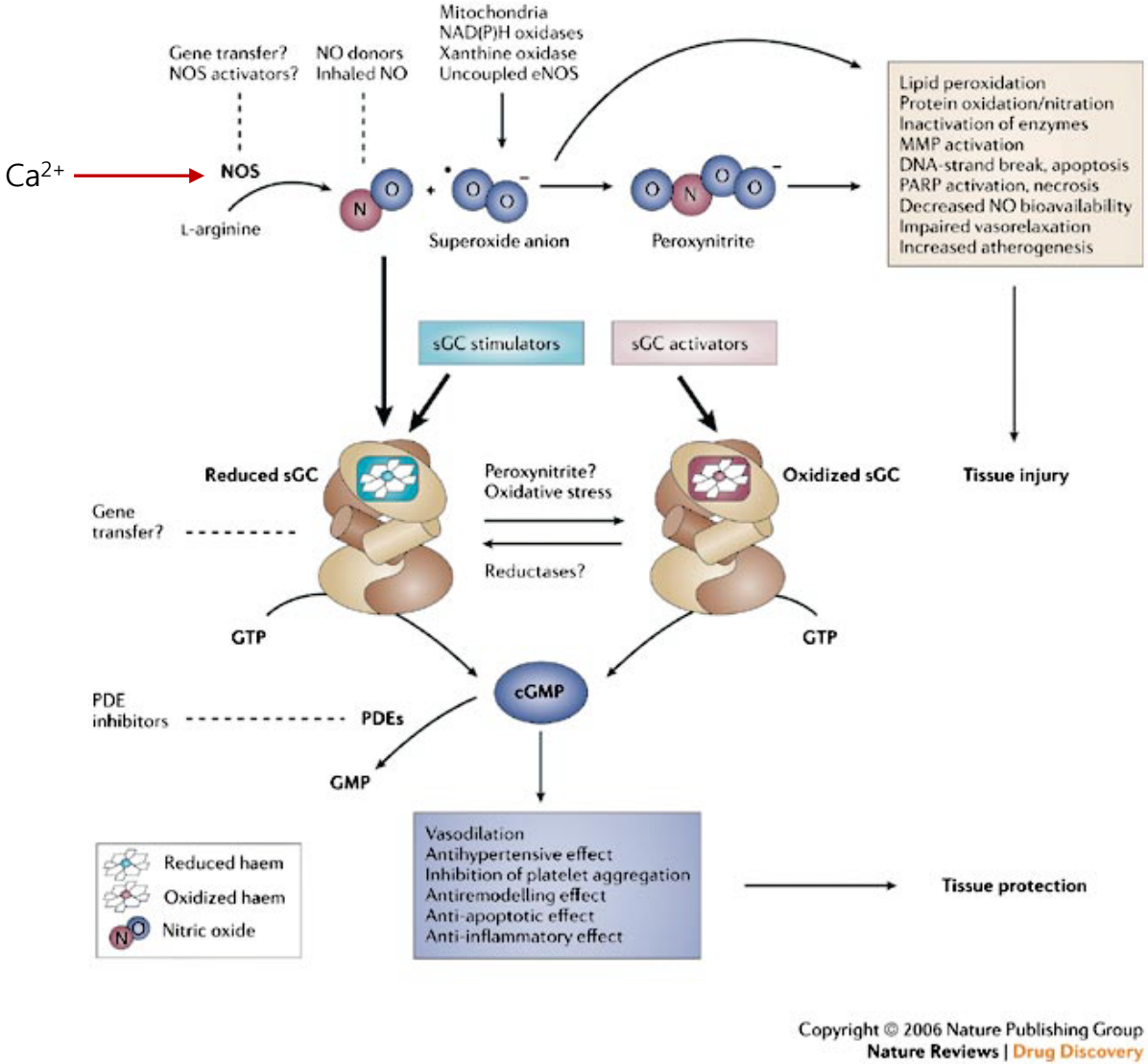


Role of adenylyl cyclase-coupled GPCRs on plasma membrane channel activity in pituitary cells. Cell Calcium (2012) 51, 212–221

Guanylyl cyclases



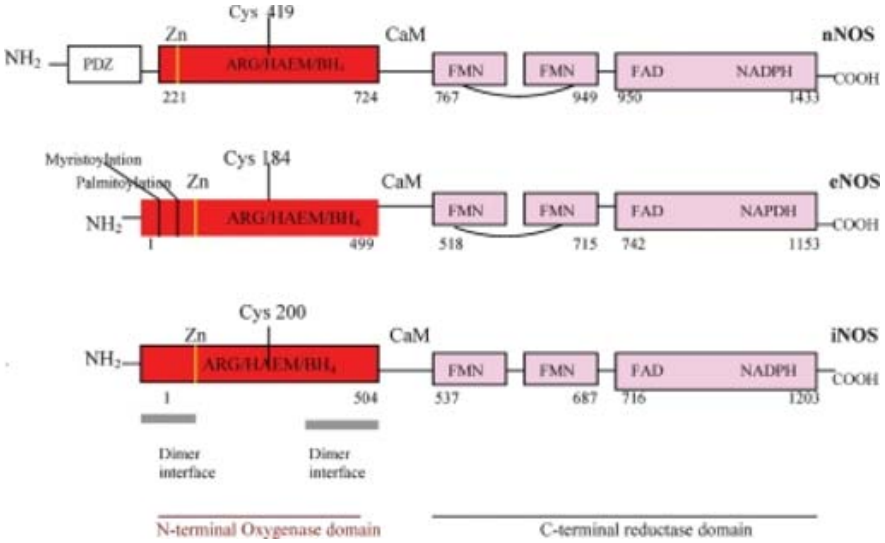
cGMP-dependent PKI, PKII
cGMP-gated ion channels
cGMP-regulated PDEs
cGK (PKG)

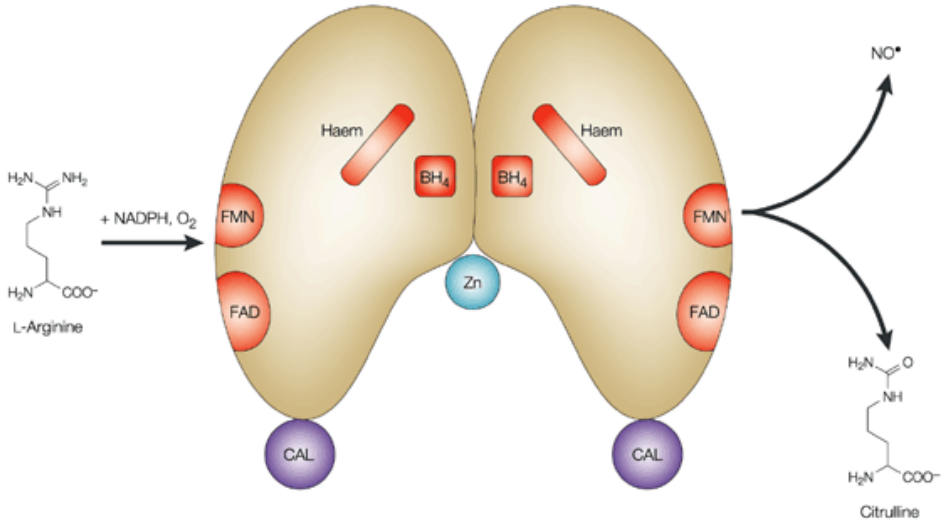


The NO–sGC–cGMP signal transduction pathway and potential drug targets. Nature Reviews Drug Discovery 5, 755-768 (September 2006)

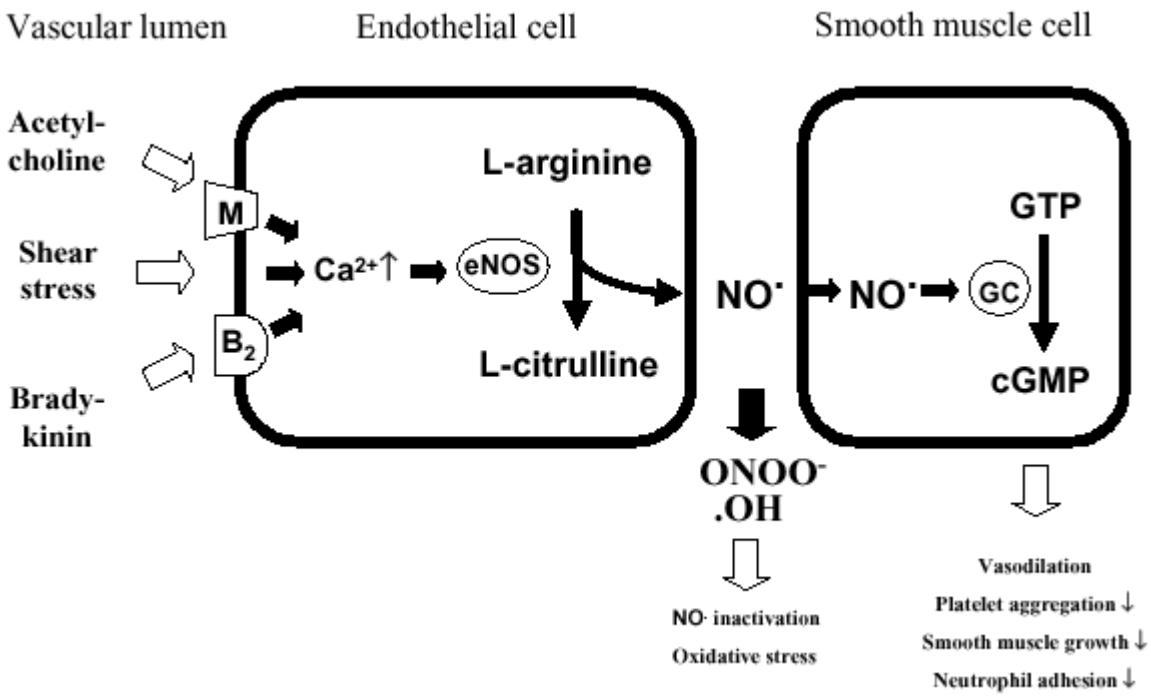
Nitric oxide synthases

nNOS (NOS1)
iNOS (NOS2): Ca^{2+}-insensitive
eNOS (NOS3)

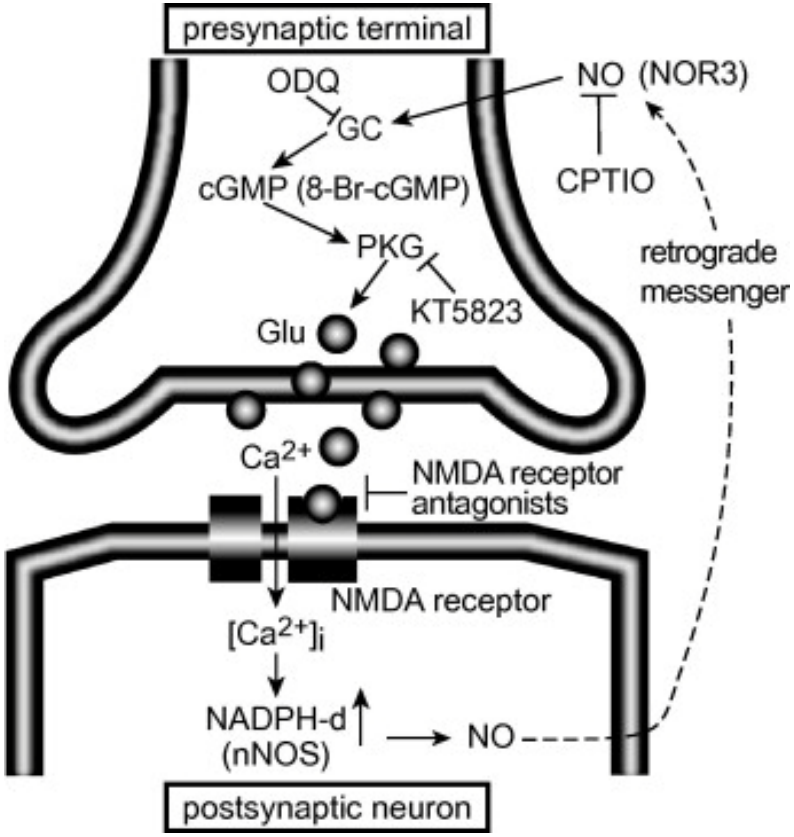




Nature Reviews | Drug Discovery

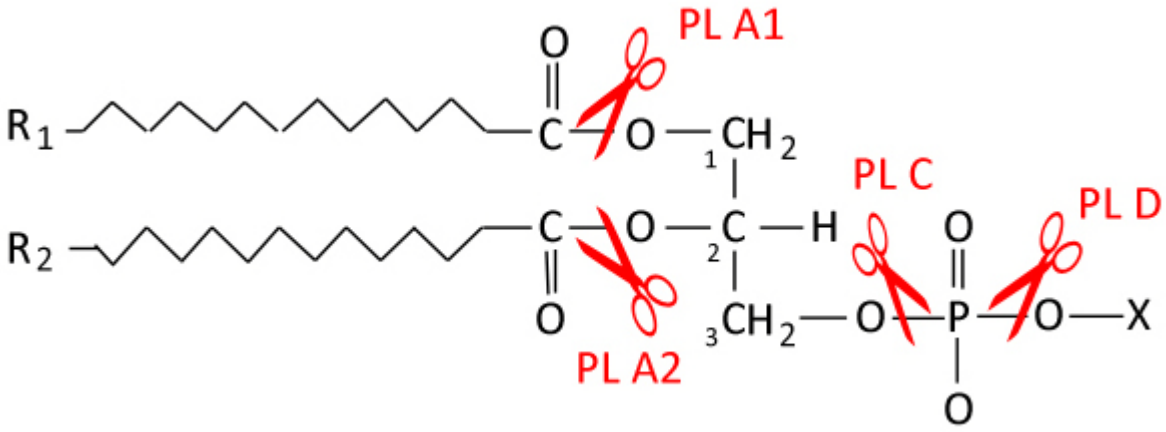

Vascular lumen
Endothelial cell
Smooth muscle cell
Acetyl-choline
Shear stress
Brady-kinin
M
B2
Ca2+↑
eNOS
L-arginine
L-citrulline
NO·
NO·
GC
GTP
cGMP
ONOO-
.OH
NO· inactivation
Oxidative stress
Vasodilation
Platelet aggregation ↓
Smooth muscle growth ↓
Neutrophil adhesion ↓

A postulated mechanism of nNOS activation
by NO as a retrograde messenger.
Nitric Oxide (2007) 17,18–24

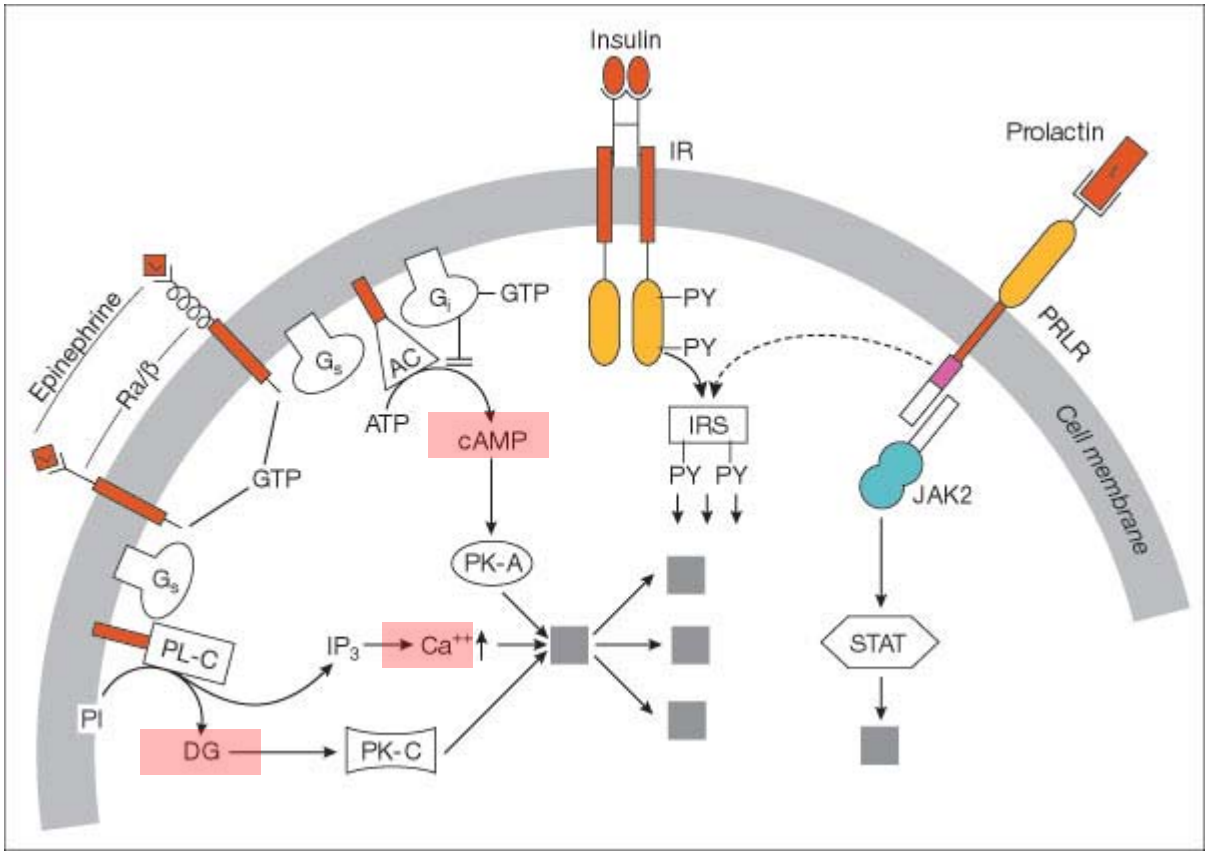


Phospholipases

PLA1
PLA2
PLC
PLD



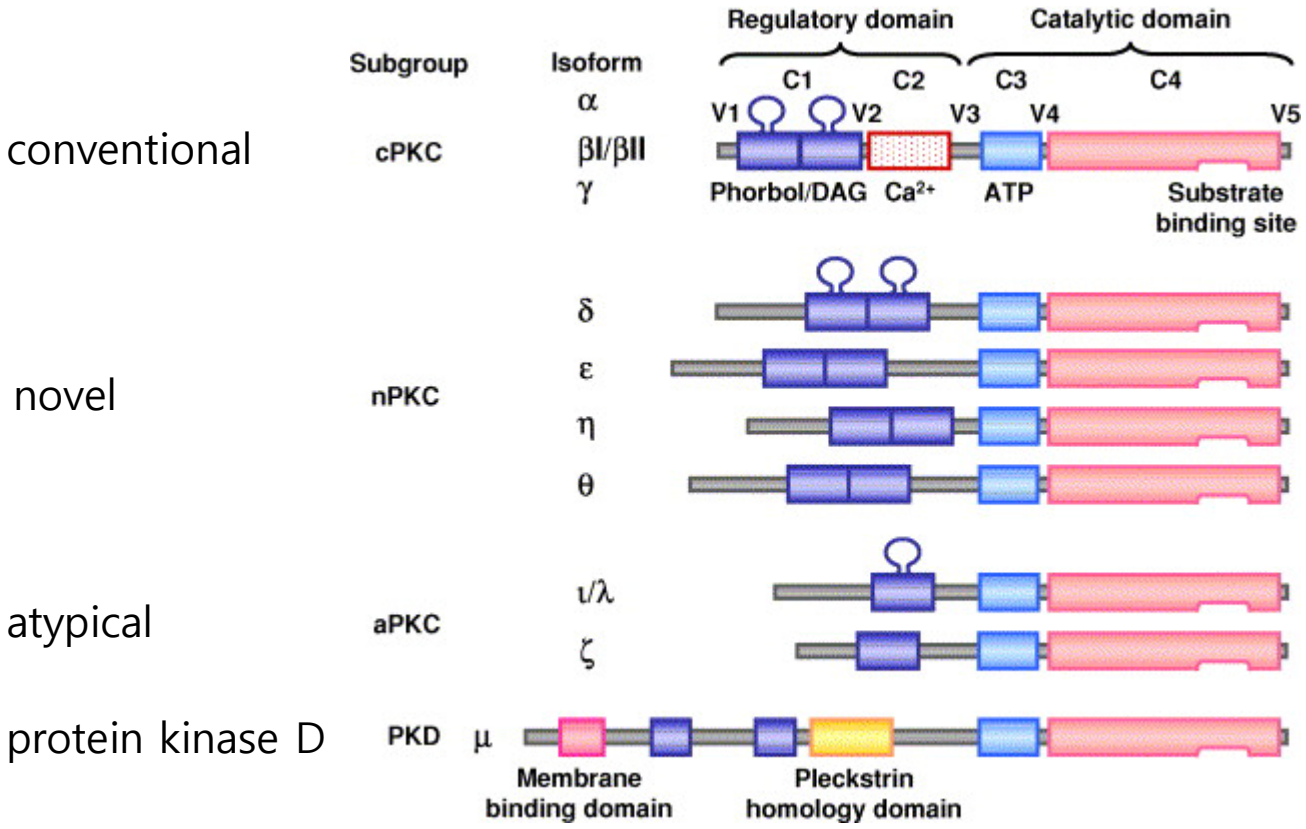
Protein phosphorylation & dephosphorylation



A simplified diagram to illustrate 'second messenger' and protein phosphorylation pathways associated with target-cell membrane receptors for three hormones: epinephrine (adrenaline), insulin and prolactin. AC, adenylyl cyclase; cAMP, cyclic AMP; DG, diacylglycerol; Gs and Gi, stimulatory and inhibitory G proteins; IP_3, inositide trisphosphate; IRS, insulin receptor substrate; JAK, Janus kinase; PI, phosphatidyl inositol; PK-A and PK-C, phosphokinases A and C; PL-C, phospholipase C; PY, phosphotyrosine; Rα/Rβ, IR and PRLR, receptors for epinephrine, insulin and prolactin, respectively; STAT, signal transduction and transcription factor. The grey squares indicate different protein targets for phosphorylation by different pathways, depending on hormonal signal, species and tissue. EMBO reports (2005) 6, 490 - 496

PKA: cAMP
PKG: cGMP
PKC: calcium ion
CaMK: calmodulin

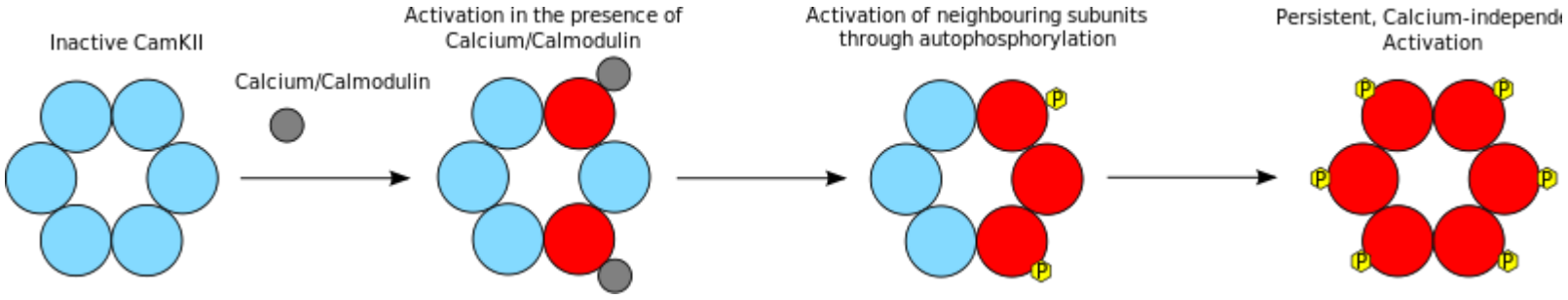
Structure of PKC isoforms



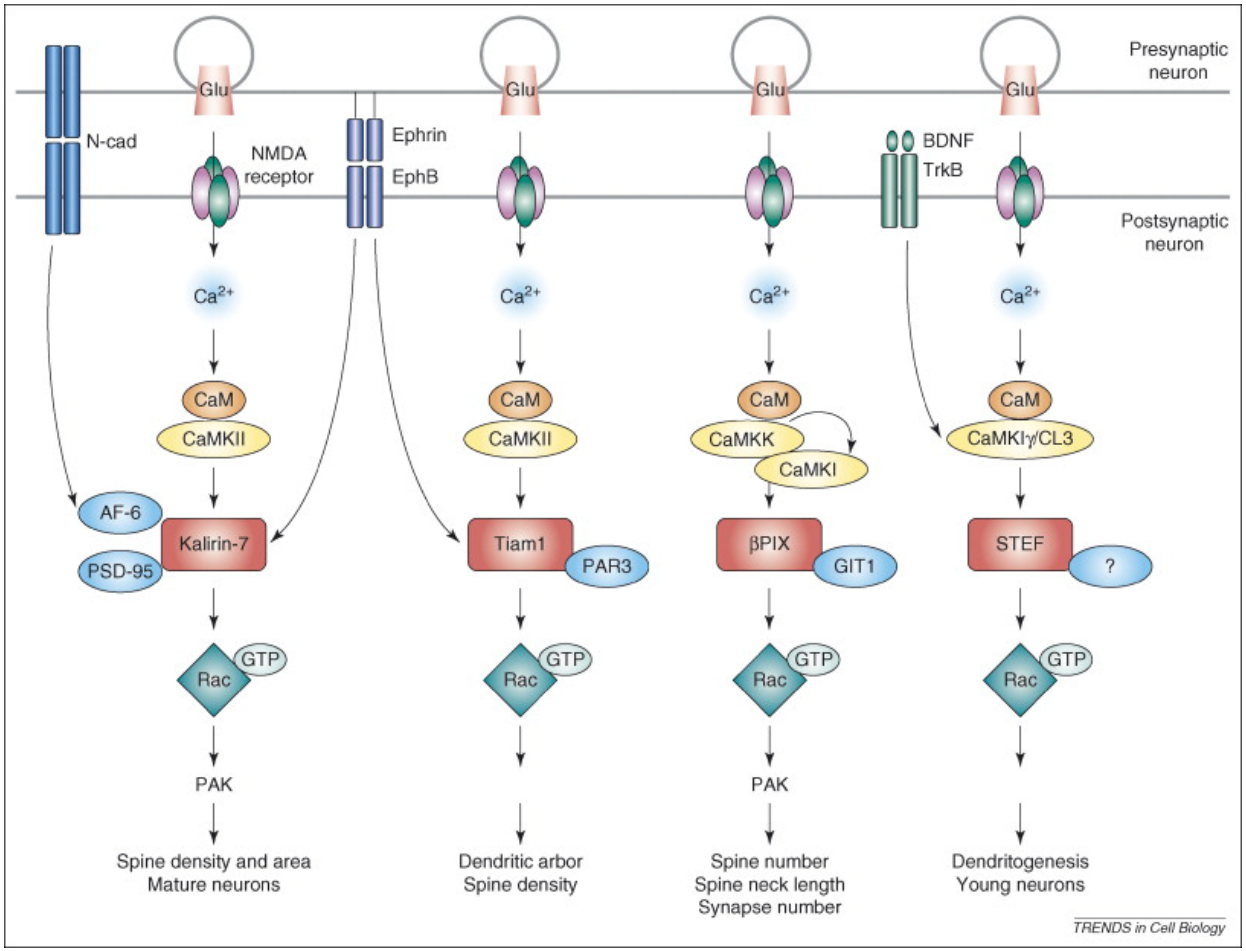
PKC is composed of four conserved (C1–C4) and five variable (V1–V5) regions. C1 region contains binding sites for DAG, phorbol ester, phosphatidylserine and the PKC antagonist calphostin C. C2 region contains the binding site for Ca2+. C3 and C4 regions contain binding sites for ATP, some PKC antagonists and different PKC substrates. The PKC molecule folds to bring the ATP binding site into proximity with the substrate-binding site. Binding of an endogenous or exogenous pseudosubstrate peptide sequence to the catalytic domain prevents PKC from phosphorylating the true substrate. Biochem Pharm. (2005) 70,1537-1547.

CaM-kinases :
CaMKII & IV: multifunctional
CaMKI: phosphorylate synapsin (?)
CaMKIII: eEF2 kinase
CaMKIV: nucleus enzyme, phosphorylate CREB
Myosin light chain kinase
Phosphorylase kinase: glycogenolysis

CaMKII: multimeric consisting of 28 different isoforms (α,β,γ,δ)
implicated in long-term potentiation



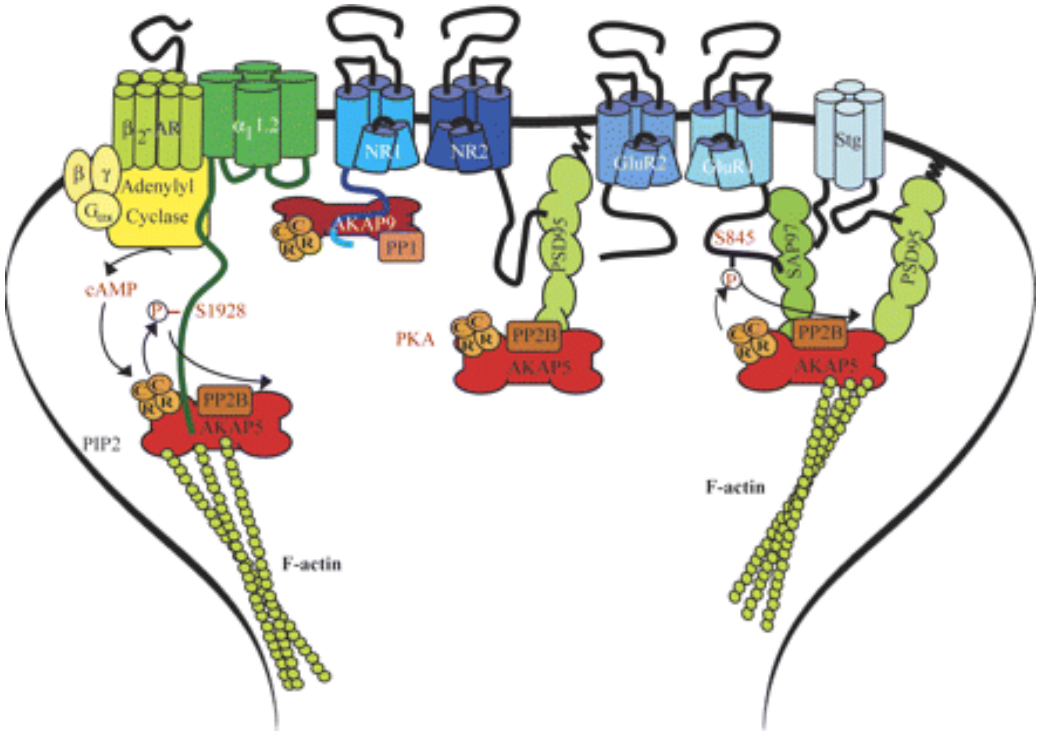
CaM-kinase (CaMK)



Comparison of CaMK–RacGEF pathways. Ca^{2+} influx occurs through activated NMDA receptor ion channels. Ca^{2+} then complexes with CaM (orange), which can activate CaMKs (yellow). CaMKs then phosphorylate downstream RacGEFs (pink), which regulate the activity of Rac through GDP–GTP exchange, thus, mediating effects on dendrites and dendritic spines by means of reorganization of the actin cytoskeleton. GEFs can be in complexes with scaffolding or other types of binding partners, such as PSD-95, afadin (AF)-6, partitioning defective 3 (PAR3) and GIT1. Other pathways, such as N-cadherin, ephrin-EphB or BDNF–TrkB (neurotrophic tyrosine receptor kinase type-2) signaling, can also modulate the activity of RacGEFs. Abbreviations: G, glutamate; N-Cad, N-cadherin. TICB (2008) 18,405-413.

Protein kinase-anchoring proteins (AKAPs): PKA, PKC

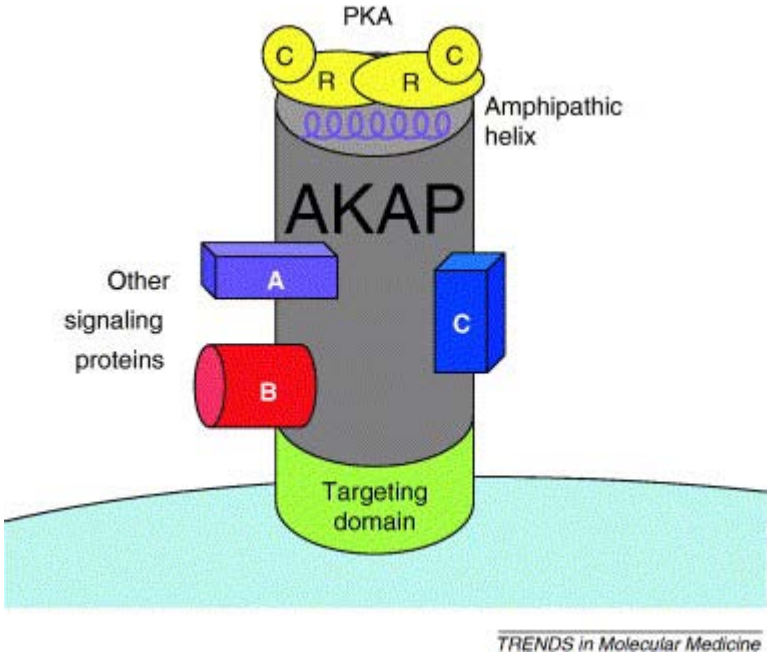
sequester protein kinases to the region of a neuron



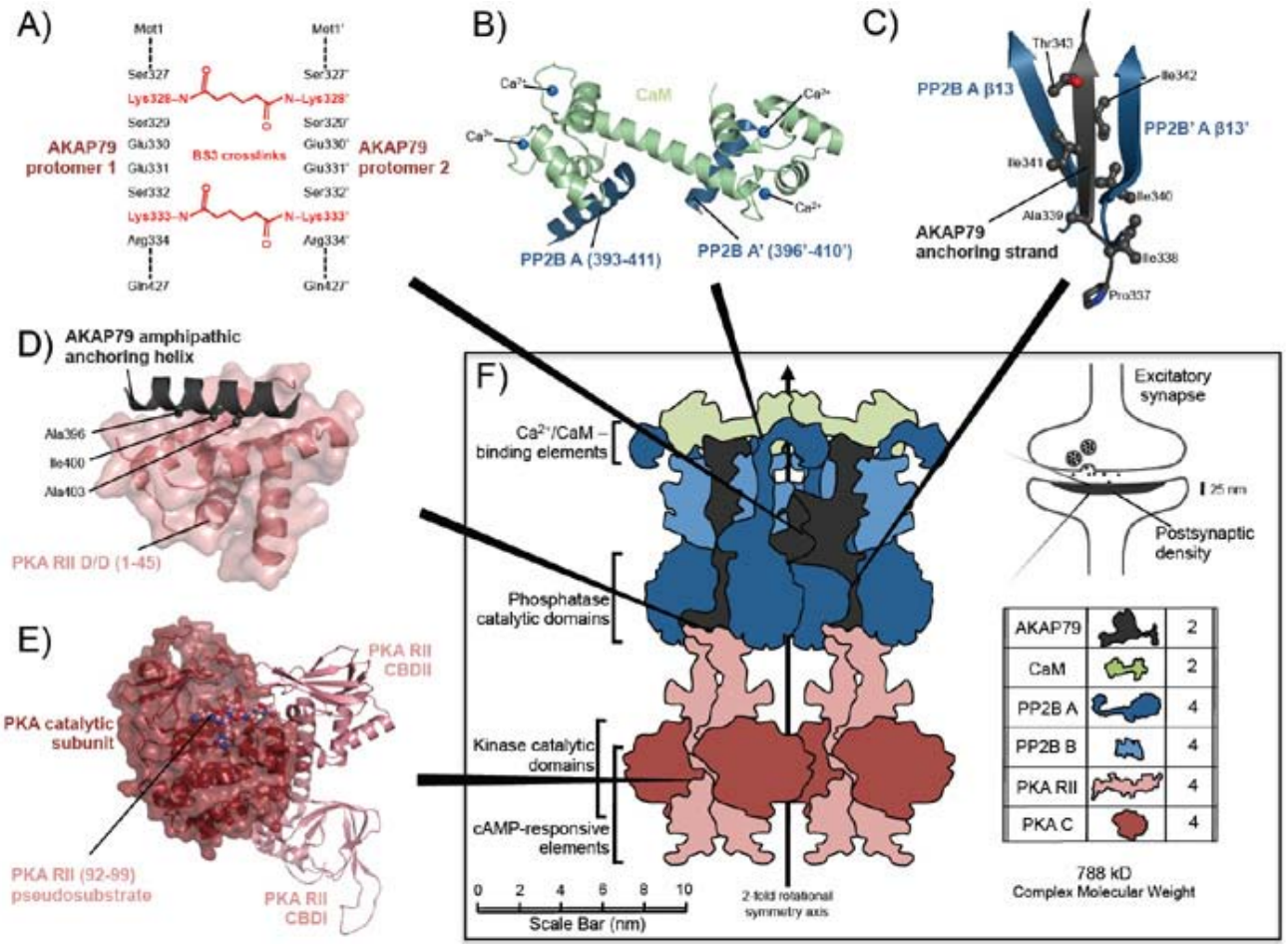
Postsynaptic A kinase anchor protein (AKAP)/PKA complexes

Physiol. Rev (2009) 89,411-452

AKAP-PKA complex

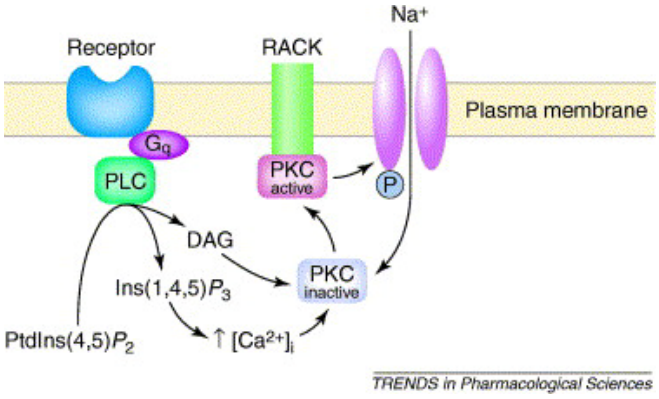


Schematic diagram of a prototypic AKAP. Anchoring of PKA (yellow) to AKAP is accomplished through hydrophobic interaction between the amphipathic α helical region of AKAP and the surface formed by the N-terminal dimerization region of the two R subunits of PKA. When cAMP binds to the R subunit, the C subunit of PKA is activated and released to phosphorylate nearby substrates. AKAP also serves as a signaling scaffold for various other signaling enzymes (A, B and C). Finally, the targeting region of AKAP (green) localizes the entire complex to the appropriate subcellular compartment via protein–protein or protein–lipid interactions. TIMM (2006) 12,317-323

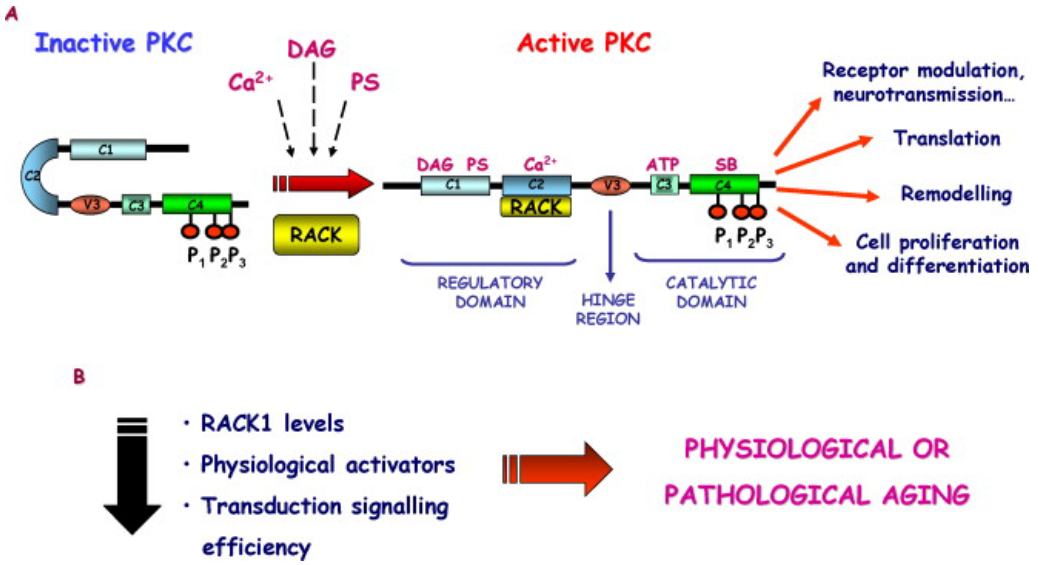


Structure of the postsynaptic AKAP79 signalling complex. AKAP79 is an A-Kinase Anchoring Proteins (AKAP). Members of this prototypical mammalian anchoring protein family position the major intracellular receptor for cAMP (PKA) in proximity to adenylyl cyclase at different intracellular sites. AKAP79 also anchors the Ca2+-sensitive phosphatase calcineurin in proximity to Ca2+ entry sites. AKAP79 directs both PKA and calcineurin for phosphoregulation of AMPA-type glutamate receptors. http://www.ucl.ac.uk/npp/research/mgg

PKC-anchoring proteins: RACKs (Receptors for activated C kinases)



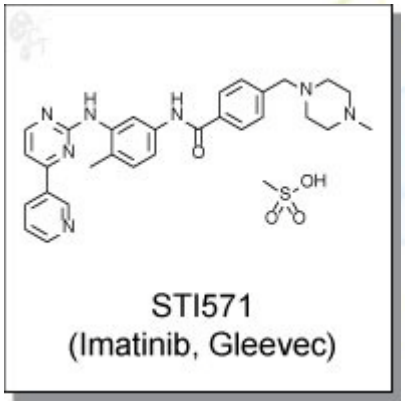
TIPS (2005) 26,496-502

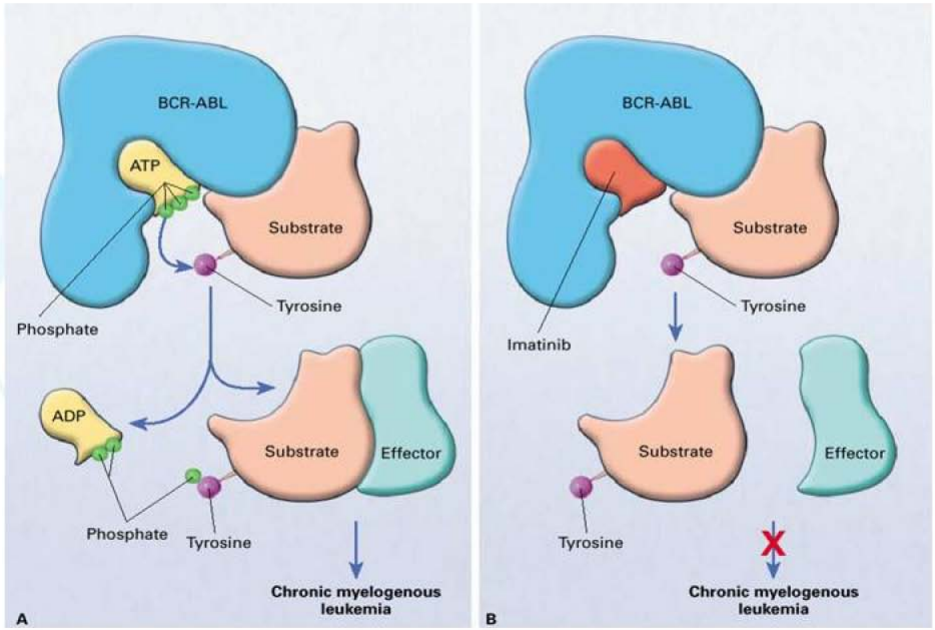


(A) DAG, Ca2+ and PS are important factors in the activation mechanism of PKC, allowing it to assume its "unfolded active conformation". The figure shows the organization of a general conventional PKC as a model (note that RACK1 interacts also with the V5 region of PKCβII-not shown [94]). The activated enzyme can then translocate to different subcellular compartments taking part to multiple cellular functions, such as neurotransmission, synaptic remodelling, cell proliferation, translation, etc. Anchoring proteins (i.e. RACK1 for PKCβII) have been documented to be also implicated in the translocation process. (B) Alterations/reductions (arrow down) in any of these actors can induce a deregulation of the mentioned pathways leading to some of the changes occurring in physiological or pathological (i.e. Alzheimer's disease) aging. Ca2+: calcium; DAG: diacylglycerol; PS: phosphatidylserine; SB: substrate; P1: phosphorylation at the "active loop"; P2: phosphorylation at the "turn motif"; P3: phosphorylation at the "hydrophobic motif". Pharm Res (2007) 55,560-569.

Protein kinase inhibitors (PKIs)

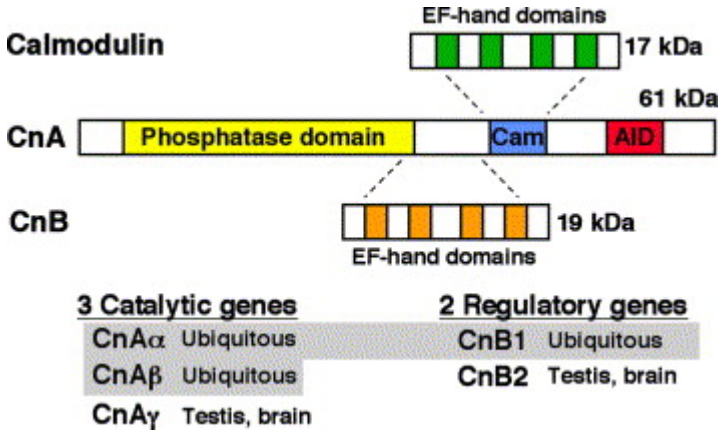
Gleevec®: Protein Kinase Inhibitor Therapy for Chronic Myeloid Leukemia



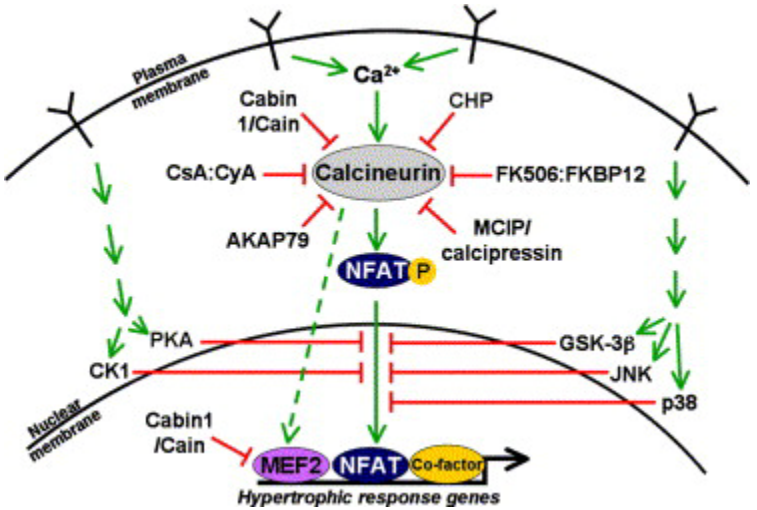


Protein phosphatases (Proten serine-threnine phosphatases)

PP2B (calcineurin) BBRC (2004) 322,1178-1191

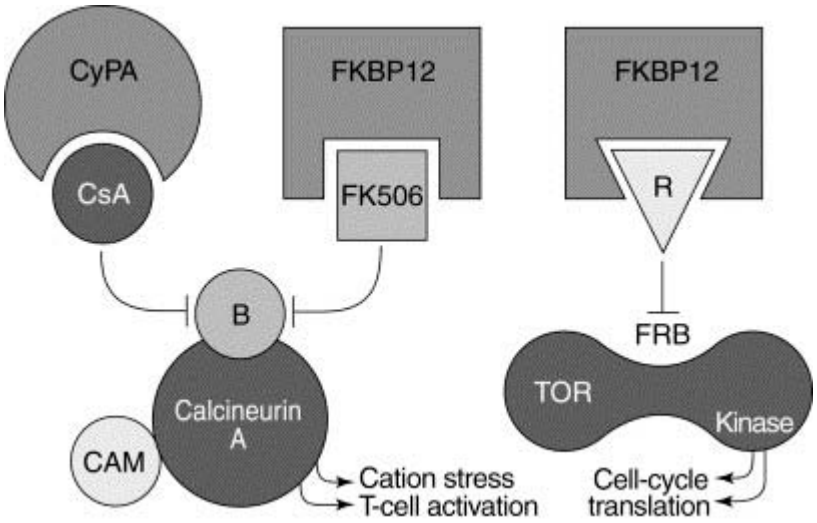


Schematic representation of the catalytic calcineurin A subunit (CnA), the regulatory calcineurin B subunit (CnB), and calmodulin. A summary of expression for the three calcineurin A genes and two calcineurin B genes is also shown (grey boxed area represents those isoforms that contribute to calcineurin activity in most vertebrate tissues). Abbreviations not defined in text: Cam, calmodulin-binding domain; AID, autoinhibitory domain.

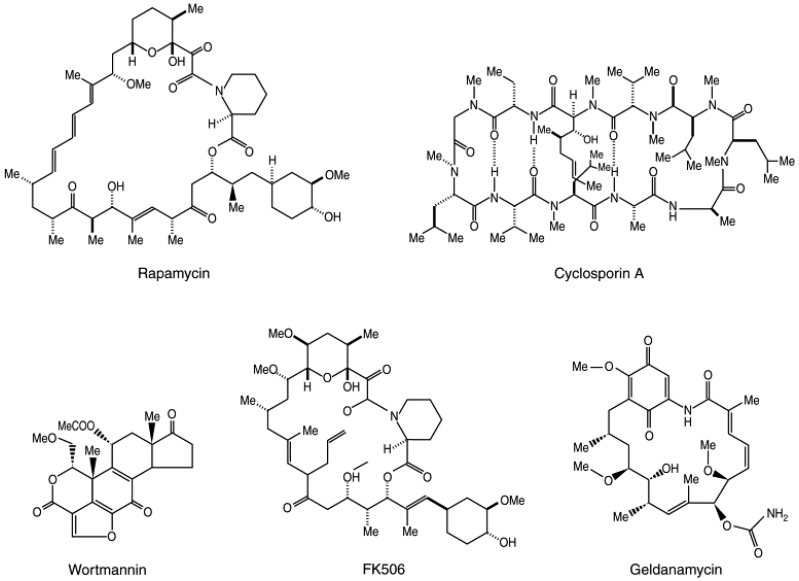


Schematic of calcineurin signaling resulting in NFAT dephosphorylation and nuclear translocation, or direct activation of MEF2 factors in the nucleus. A number of key calcineurin inhibitory factors are shown in the cytoplasm, while signal transduction through other pathways activates an array of kinases that serve to re-phosphorylate NFAT, either antagonizing its nuclear translocation or facilitating its nuclear egress. Green lines denote activation events while red lines denote inhibitory events. Abbreviation not defined in text: CyA, cyclophilin A.

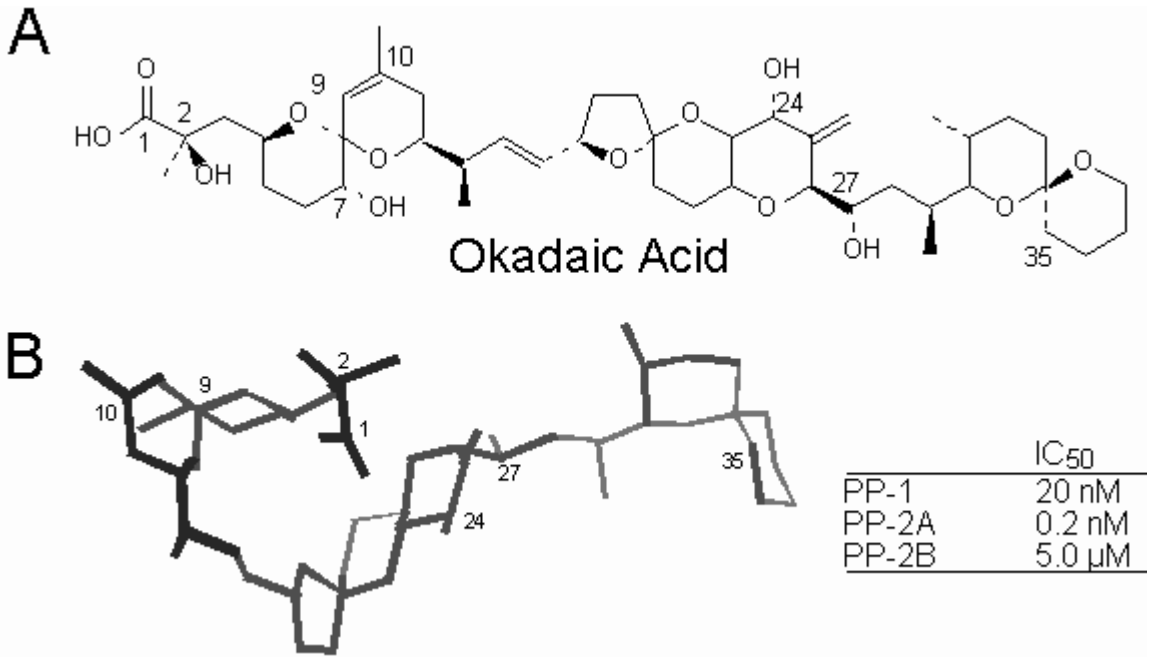
Signal-transduction cascades as targets for therapeutic intervention by natural products. TIBT (1998) 16,427-433.



Cyclosporin A (CsA), FK506 and rapamycin (R) form complexes with the highly conserved **prolyl-isomerase immunophilin proteins cyclophilin A (CyPA) and FKBP12**. The resulting CyPA–CsA and FKBP12–FK506 complexes then bind to and inhibit the protein phosphatase calcineurin, a heterotrimer of the catalytic (A) and regulatory (B) subunits and calmodulin (CAM) that transduces signals during the response of yeast cells to cation stress and during T-cell activation. The FKBP12–rapamycin complex binds to the TOR-kinase homologues at the FKBP12–rapamycin-binding domain (FRB). The TOR kinase transduces growth-promoting signals that are sent in response to nutrients and growth factors, and drive cell-cycle progression, possibly by regulating translation.



Molecular structures of some natural products that inhibit signal transduction. The molecular structures of the immunosuppressants cyclosporin A, FK506 and rapamycin, and of the antiproliferative agents wortmannin and geldanamycin are depicted. Cyclosporin A is a cyclic peptide, FK506 and rapamycin are related macrolide antibiotics, wortmannin is steroid-like, and geldanamycin is a benzoquinone ansamycin.



	IC_{50}
PP-1	20 nM
PP-2A	0.2 nM
PP-2B	5.0 μM

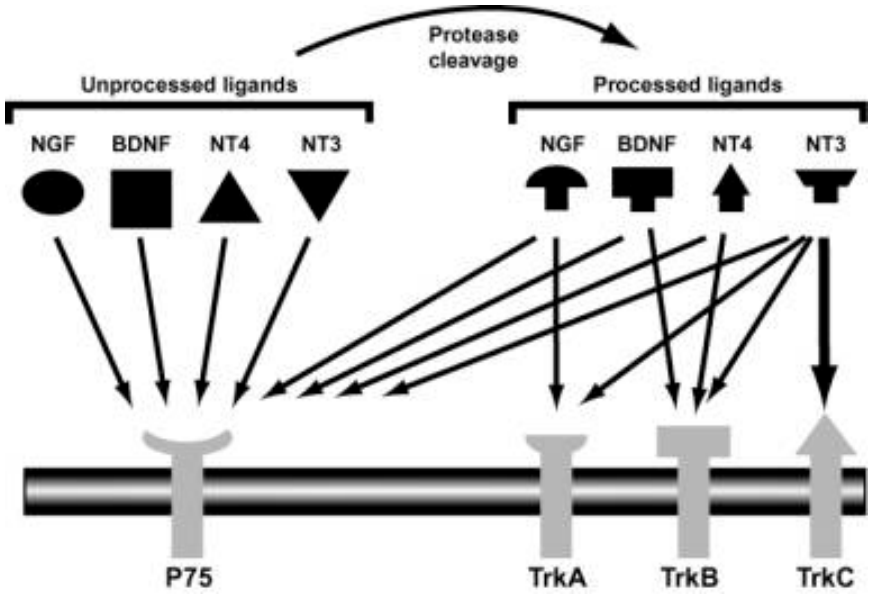
Okadaic acid was named from the marine sponge Halichondria okadai, from which okadaic acid was isolated for the first time.[1] It has also been isolated from another marine sponge, H. malanodocia, as a cytotoxin. The real producers of okadaic acid belong to the algae group of the dinoflagellates, namely the benthic dinoflagellate Prorocentrum and the planktonic forms of Dinophysis, as for example Dinophysis acuminata.

Neurotrophins

A family of proteins that induce the survival, development, and function of neurons.

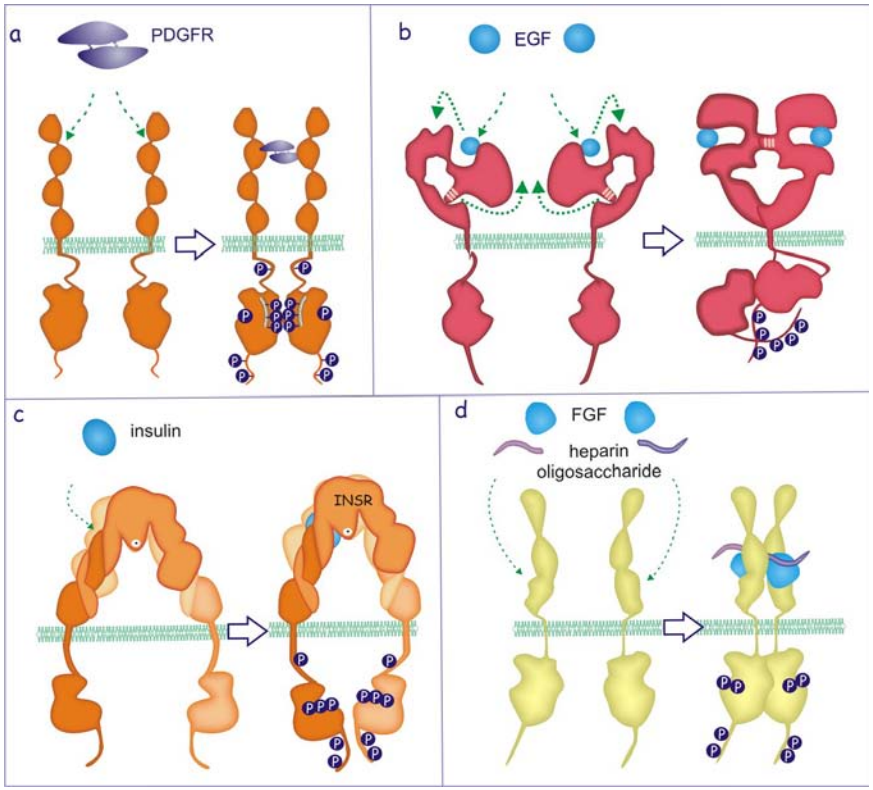
Nerve growth factor (NGF)
Brain-derived neurotrophic factor (BDNF)
Neurotrophin-3 (NT-3)
Neurotrophin-4 (NT-4)

Mostly involve the activation of protein tyrosine kinases



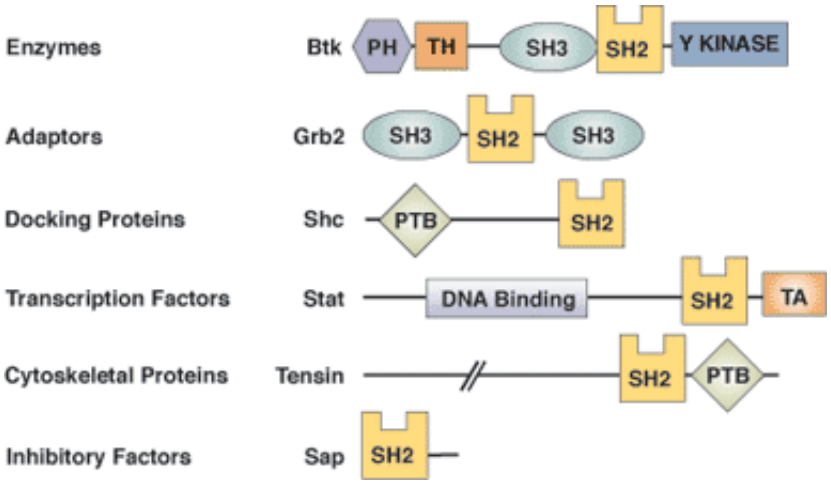
The Trk (tropomyosin receptor kinase) receptor was first identified as a colon-derived oncogene in which tropomyocin was fused to a tyrosine kinase domain. However, the normal cellular counterpart of this oncogene, namely Trk (lacking tropomyocin), was found to be a transmembrane protein that is highly expressed in the developing and adult nervous system. To date, three Trk receptors have been identified, TrkA (or NTRK1), TrkB (or NTRK2), and TrkC (or NTRK3), each of which has a different neurotrophin binding specificity: TrkA binds NGF, TrkB binds BDNF and NT4, and TrkC binds NT3 (TrkA and TrkB bind NT3 to a lesser extent). The neurotrophins are synthesised in a precursor form (pro-neurotrophins) that are proteolytically cleaved to generate mature neurotrophins. These pro-neurotrophins bind to p75NTR with a higher affinity than mature neurotrophins, but bind only weakly to Trk receptors. Therefore, p75NTR can bind all the neurotrophins in their precursor form, while the Trk receptors are selective for specific mature neurotrophins.
http://www.ebi.ac.uk/interpro/potm/2005_8/Page.htm

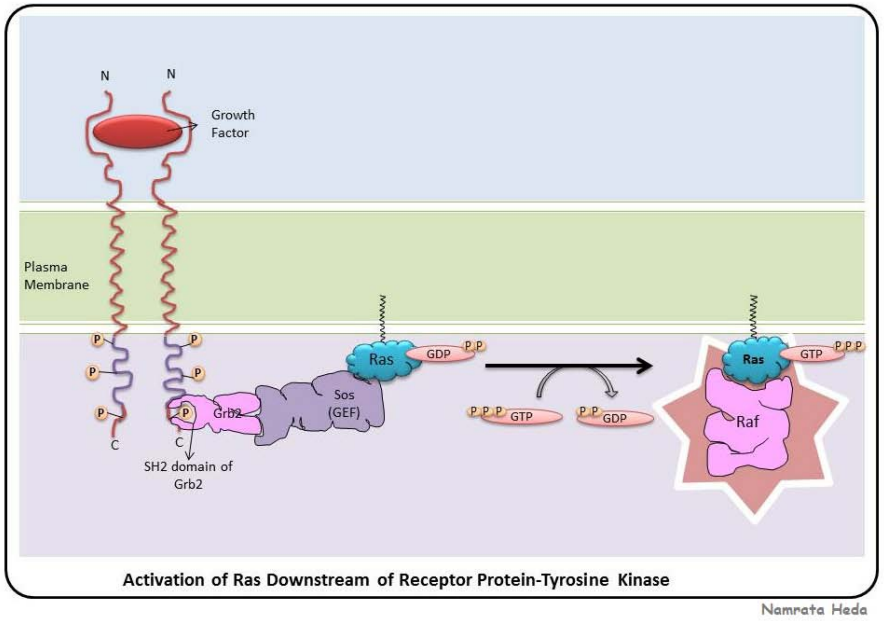
Protein receptor kinases



Receptors employ different dimerization strategies.
a) PDGF forms a ligand dimer of which each growth factor engages one receptor; b) EGF has one binding site and its binding reveals a receptor dimerization motif; c) insulin has two binding sites and its action somehow must change the conformation of an existing receptor dimer; d) FGF has two binding sites but two ligands are needed to bring two receptors together. Stable dimers only form when two heparin sulphate oligosaccharides combine with receptor ligand complexes.

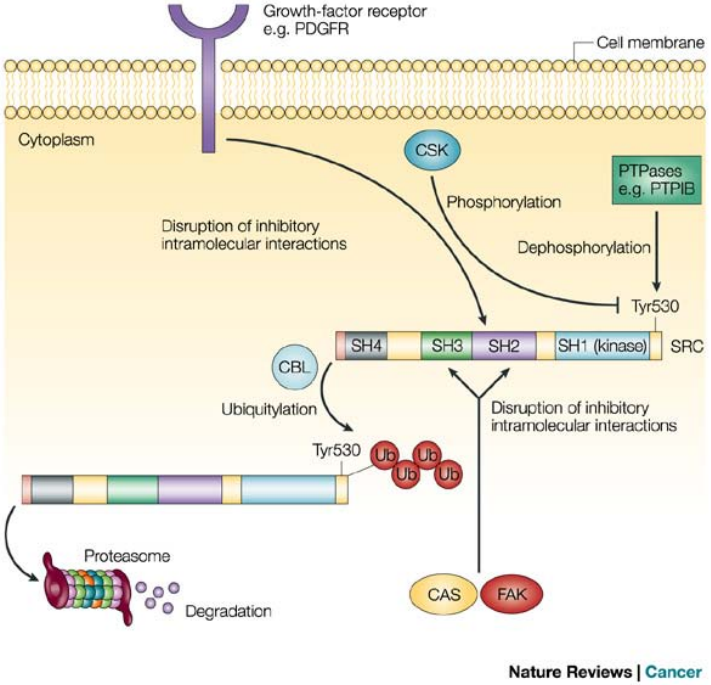
Phospho/Tyr Binding: SH2 domain

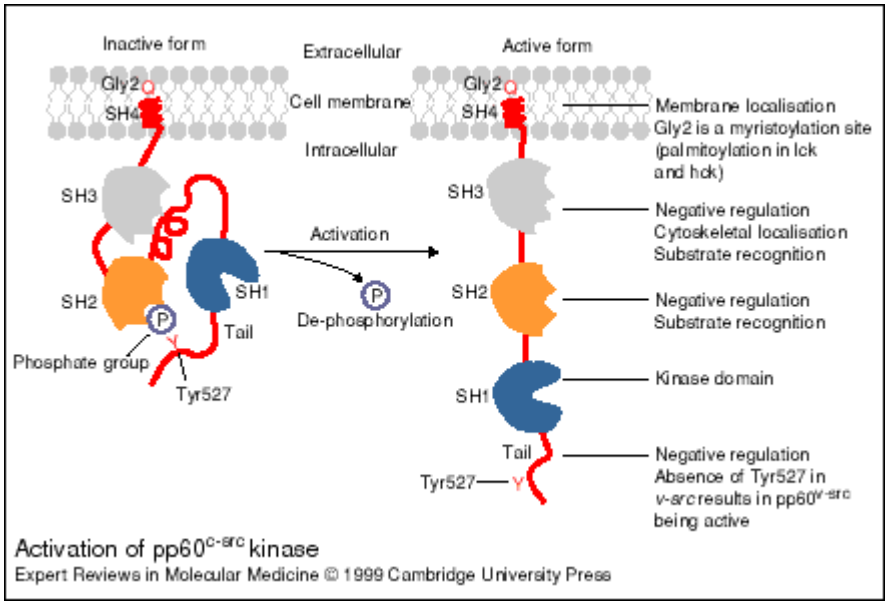




Src-homology 2 (SH2) domains are modules of approximately 100 amino acids that bind to specific phospho (pY)-containing peptide motifs. Conventional SH2 domains have a conserved pocket that recognizes pY, and a more variable pocket that binds 3-6 residues C-terminal to the pY to confer specificity. The SAP SH2 domain recognizes Y as well as pY in the context of residues N and C terminal, suggesting that an alternate 3-pronged model may sometimes apply. Phosphopeptides of optimal sequence bind to SH2 domains with dissociation constants of ~50-500 nM.
http://www.cellsignal.com/reference/domain/sh2.html

c-Src (c-Src tyrosine kinase): cytoplasmic TK





Inactivation of c-SRC is carried out by c-SRC tyrosine kinase (CSK), which phosphorylates a conserved tyrosine residue in the c-SRC carboxy-terminal domain (Tyr530). This is reversed by phosphatases such as protein tyrosine phosphatase 1B (PTP1B), which leads to c-SRC activation. Activation of growth-factor receptors leads to their association with the c-SRC SRC homology 2 (SH2) domain, which disrupts inhibitory intramolecular interactions to promote c-SRC activation. Other proteins, such as CRK-associated substrate (CAS) and FAK, bind to the c-SRC SH2 and SH3 domains to promote c-SRC activation by a similar mechanism. Levels of c-SRC protein are negatively regulated by the E3 ubiquitin ligase CBL, which leads to c-SRC ubiquitylation and subsequent degradation by the proteasome. Nature Rev Cancer (2004) 4, 470-480

JAK-STAT signaling pathway

JAK (Janus kinase): cytoplasmic TK
JAKs possess two near-identical phosphate-transferring domains. One domain exhibits the kinase activity, while the other negatively regulates the kinase activity of the first.

STAT (Signal transducer and activator of transcription)

Transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell.

The interaction of a cytokine (black) with its specific receptor (dark green) induces receptor-complex dimerization. This brings members of the JAK cytoplasmic kinases (yellow) into juxtaposition, allowing them to transphosphorylate each other and to phosphorylate specific STAT proteins (pale green). STATs are found in the cytoplasm in monomeric form and, following tyrosine-residue phosphorylation by the activated JAKs, they associate with the cytokine receptor, homo- or hetero-dimerize, translocate to the nucleus and bind to specific DNA elements (STAT-recognition sites) that are situated upstream of the genes whose expression is induced by cytokines. TINS (1999) 22,365-369.