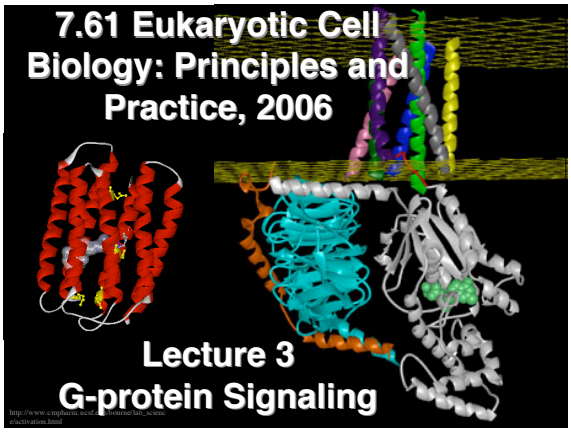
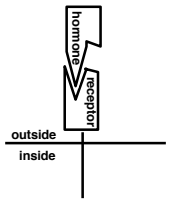
7.61 Eukaryotic Cell Biology: Principles and Practice, 2006



Lecture 3
G-protein Signaling

Hydrophilic Ligand/Receptor System

Extracellular Binding Requires Signal 'Transduction'



7-TM or G-Protein Coupled Receptors (GPCR)

Majority of transmembrane signaling via hormones, neurotransmitters, sight and smell mediated by GPCRs

About 2000: ~5% of worm and 3% of mammalian genomes

~2000 reported since 1st cloning (1983, bovine opsin). The human genome is now known to encode approximately 1000 GPCRs, ~ 400 non-olfactory receptors; ~150 orphan receptors (~2003); in last 17 years ~15 de-orphanized.

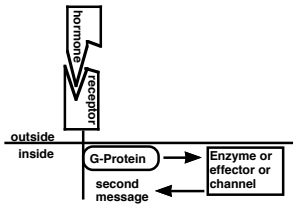
7-TM or G-Protein Coupled Receptors (GPCR)

3 major families: rhodopsin, calcitonin, and metabotropic. β-adrenergic receptor and rhodopsin 1st purified an characterized.

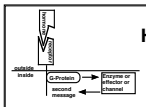
Major Drug Targets for Pharmaceutical Industry

- **50% of all modern drugs (~20% of the top 50 best-selling drugs) are targeted at GPCRs (including adrenergic, histaminergic, dopaminergic, serotonergic, opiate, cholinergic, etc.) for pain, asthma, inflammation, obesity, cancer, and cardiovascular, metabolic, gastrointestinal and CNS diseases (e.g., Claritin, Zyprexa, Zantac and Cozaar).**

Focus on G-Protein Coupling
(there are others)



Adenylate Cyclase System
Hydrophilic hormone/Surface Receptor
ATP->cAMP
Altered cellular metabolism
Some hormones induce, some suppress cyclase



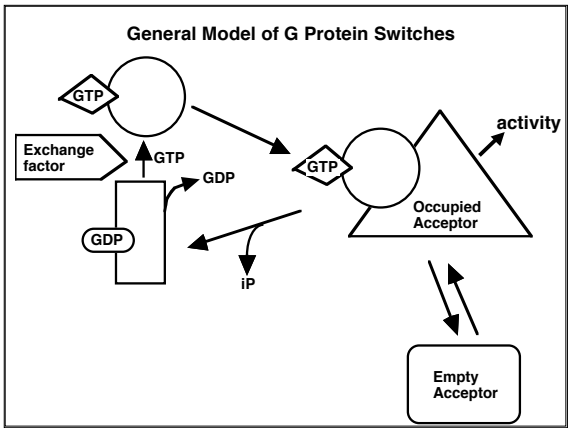
How do we know what we know?

We will start from the point at which it was clear that there were distinct adrenergic receptors (β,α) and glucagon receptors, and an adenylate cyclase activity that was activated by glucagon & β adrenergic receptors and inhibited by α adrenergic receptors.

1971 Nobel Prize to Earl Sutherland for second message cAMP

1994 Nobel Prize: Al Gilman & Martin Rodbell (1971) for discovering and working out G-protein mechanism

"In World War II [Rodbell] had been in radio communications, typing out Morse code relayed to him through earphones. Little did he know then the importance that signal transduction would play in his life!" L. Birnbaumer, http://www.sciencemag.org/cgi/content/full/283/5408/1656



Experimental findings which have lead to the current model:

1. GTP enhanced hormone activation of cyclase and reduction of hormone (glucagon) binding to receptors in membrane preps. (Rodbell, 1960s) - contaminant of ATP!
2. GTP reduces agonist, but not antagonist binding to receptors (coupling important)
3. Nonhydrolyzable GTP analogues (GPPNP) work: (hydrolysis not required)
4. Nonhydrolyzable GTP analogues give permanent activation (a GTPase turns off signal)
5. ß-adrenergic receptor-dependent slow GTPase (evidence for direct role of GTP)
6. GTP binding protein purified via detergents by GTP affinity column away from cyclase, reconstitutes agonist-dependent cyclase in S49 cyc^- membranes (receptors and cyclase, but not coupled) and GPPNP-dependent cyclase activity (separate subunits) - this is first direct evidence of 3 components

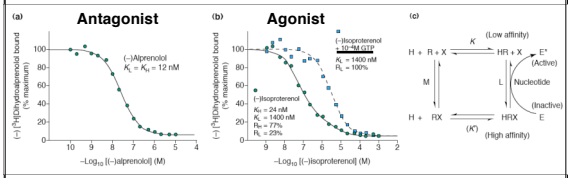
7. Identification of GTP-binding subunit by [^{32}P]GTP-derived affinity label and by cholera toxin (which activates system)-catalyzed [^{32}P]ADP-ribosylation of GTP-binding subunit.
8. Purification of receptor and cyclase came after G protein (why?), but finally all reconstituted into liposomes, fully hormone-sensitive cyclase system requires lipid bilayer.
9. Agonist-dependent release of [^{3}H]GDP and binding of [^{35}S]GTPγS (agonist-bound receptor opens up G-binding site allowing exchange of GDP and GTP); Kds for various GTPases are 10^{-11}-10^{-7} M! very tight binding relative to ambient conc. [GTP]>10^{-4}, [GDP]>10^{-5}M; thus, not engineered to respond to GNP levels, not regulated by ratio of GTP:GDP
10. Kinetics of receptor/G interaction are cyclase independent (R/G & G/C independent).

11. Rate constant of activation of Gs-C complex linearly depends on concentration of the agonist-bound receptor : [k(on-observed) = kon(intrinsic) x $[R]_{total}$ x [H]/(K_H+[H]) ; $[R]_{total}$=total receptor; [H]=agonist concentration; K_H=dissociation constant] **receptor acts as a catalyst not permanently associated with activated complex: "collision coupling"**

12. Gs-GDP forms complex with agonist-bound receptor, dissociates when GDP exchanged with GTP or analogue; this dissociation estimated to give 10x amplification of agonist signal in addition to 100-fold amplification due to slow GTPase activity and high activity of cyclase (similar to light and rhodopsin)

13. Receptor-Gx(GDP) complex has higher affinity for agonists than receptor alone. AFFINITY OF RECEPTOR FOR ANTAGONIST NOT AFFECTED BY GTP OR ANALOGUE; GTP binding reduces affinity of R for agonist and dissociates R-G complex, permits recycling of R, lifetime of G-GTP complex before hydrolysis is many seconds ($G\alpha^{GTP}$ $t_{1/2}$~10 sec)

Competition of the binding of the β-adrenoceptor antagonist radioligand [^{3}H]dihydroalprenolol to membranes from frog erythrocytes by (a) (–)alprenolol and (b) (–)isoproterenol



X= G protein

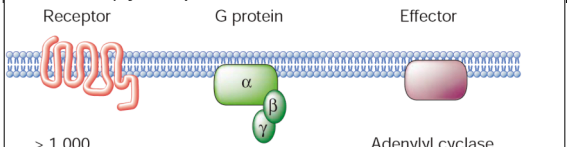
Kent RS, De Lean A, Lefkowitz RJ. A quantitative analysis of beta-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. Mol Pharmacol. 1980 Jan;17(1):14–23.

PARTS LIST:

Receptors

"G" proteins are heterotrimers:
α, 39-52 kD, β, 35-36 kD; γ, 8 kD

Effectors (cyclase)



> 1,000
Adrenergic
Dopaminergic
Opioid
Sensory – rhodopsin, olfactory, taste
Glycoprotein hormones
Lipids and other small molecules

15 α-subunits
6 β-subunits
12 γ-subunits

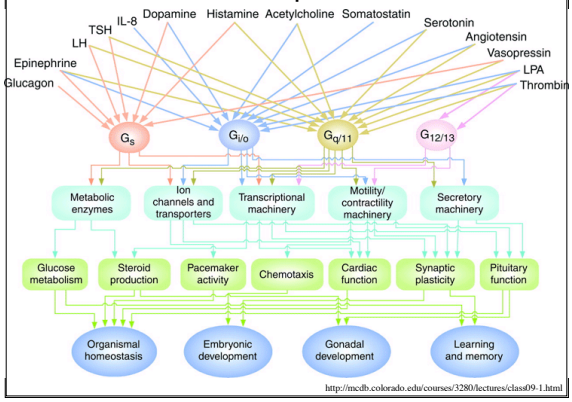
Adenylyl cyclase
Phospholipases C & A_2
cGMP phosphodiesterase
Potassium channels
Calcium channels

Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. Nature. 2002 Jan 10;415(6868):206-12 http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v415/n6868/full/415206a_fs.html

Table 1 | **Examples of heterotrimeric G-protein effectors**

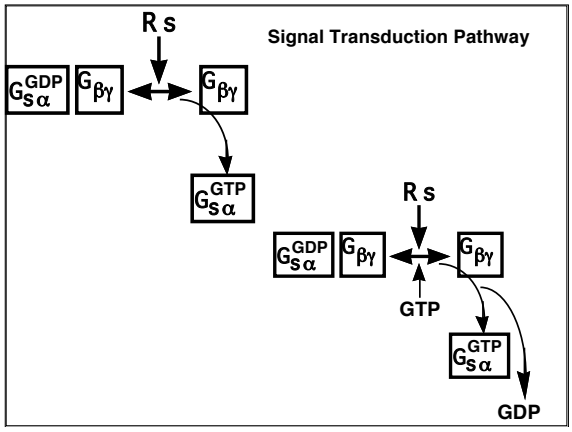
G-protein subunits	Effectors
$G\alpha_s$ $G\alpha_{olf}$	↑Adenylyl cyclase RGS-PX1 (GAP, sorting nexin) Calcium channels c-Src tyrosine kinases
$G\alpha_t$ (transducin) $G\alpha_{gust}$ (gustducin)	↑cGMP phosphodiesterase Phosphodiesterase (bitter, sweet taste)
$G\alpha_{i\ 1,2,3}$ $G\alpha_o$ $G\alpha_z$	↓Adenylyl cyclase, ↑c-Src tyrosine kinases Rap1GAP1
$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14,15,16}$	↑Phospholipase C LARG RhoGEF
$G\alpha_{12}$, $G\alpha_{13}$	p115 RhoGEF, PDZ-RhoGEF, LARG RhoGEF (Rho activation, stress-fibre formation) E-Cadherin (β-catenin release)
Gβγ	KIR3.1–3.4 (GIRK K^+ channels) GRKs ↑Adenylyl cyclases (ACII, ACIV) ↑Phospholipases (PLC β1, β2, β3) PI3Kγ

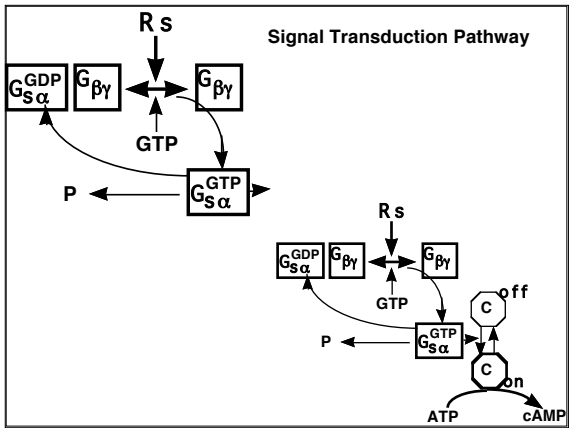
Four main G-alpha Familes

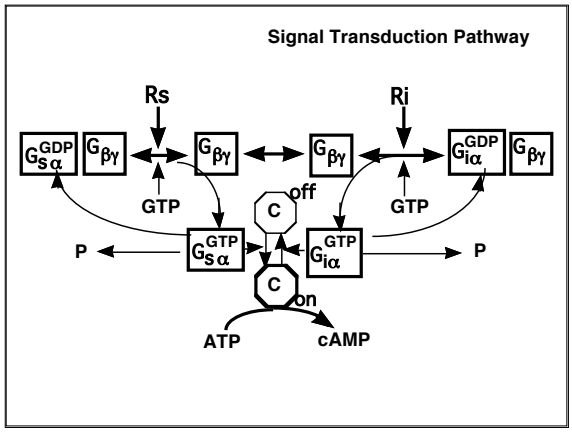


http://mcdb.colorado.edu/courses/3280/lectures/class09-1.html

Signal Transduction Pathway







G Protein's Features

G_α is GTP binding protein and GTPase
GTP hydrolysis used as a timing device
For ß-AR: H:R ($t_{1/2}$>1 sec), $G\alpha^{GTP}$ $t_{1/2}$~10 sec

$k_{catGTPase}$ (for Gα)~2-4/min-slow!, but much faster than ras: $k_{catGTPase}$ (ras)~0.02/min, almost off.....
thus, ras needs GTPase Activating Protein: GAP

$k_{diss\ GDP}$(ras)~0.008/min - about the same as $k_{diss\ GDP}$(Gαγβ complex)~0.01/min; both need GNRP (G nucleotide releasing protein), more commonly called GEF (exchange factor).

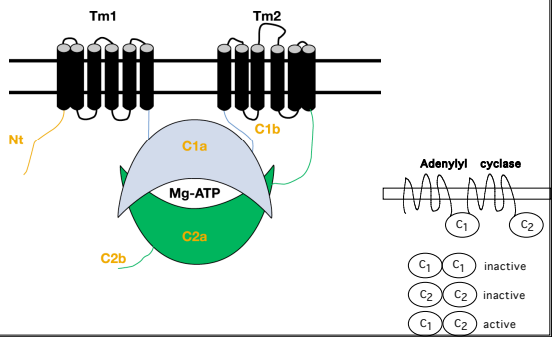
βγ subunits:
GDP dissociation from Gαγβ complex is 100x slower than for α subunit alone.....
thus, βγ keeps system inactive until hormone hits the receptor

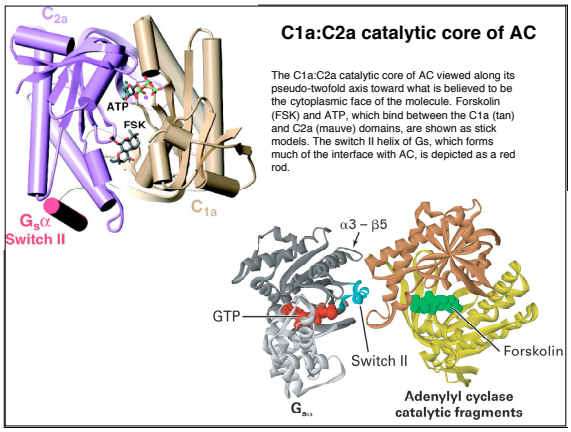
βγ required for Gα interaction with the receptor and for other regulatory steps

GαGDP can be thought of as inhibitor of βγ

Adenylate Cyclase Structure:

Tandem sets of TM and catalytically active domains





C1a:C2a catalytic core of AC

The C1a:C2a catalytic core of AC viewed along its pseudo-twofold axis toward what is believed to be the cytoplasmic face of the molecule. Forskolin (FSK) and ATP, which bind between the C1a (tan) and C2a (mauve) domains, are shown as stick models. The switch II helix of Gs, which forms much of the interface with AC, is depicted as a red rod.

Multiple adenylate cyclases:

Adenylyl Cyclase I-IX, (all Gsα stimulated)

Type I: Ca/calmodulin-sensitive, α_s stimulates, $\beta\gamma$ inhibits indirectly in presence of α_s
Type II: Ca/CaIM insensitive, α_s stimulates, $\beta\gamma$ +α_s stimulates!, PK-C regulated
Type III: little sensitivity to $\beta\gamma$
Type IV: similar to type II
Type V, VI: not regulated by $\beta\gamma$
Type VII: shares sequence with II&IV& βARK etc. associated with $\beta\gamma$ regulation

Non-classical, cytoplasmic (not integral membrane) bicarbonate-activated AC

Cyclase regulation (AC1-AC9)

Gsα All are stimulated

Giα Gi Gi, Gz, Go inhibit calmodulin- and FSK-stimulated AC1 activity; Gi inhibits AC5 & AC6; No effect on AC2

Gβγ AC2, AC4, & AC7 stimulated; AC1 inhibited; AC5 & AC6 maybe inhibited by the β1γ2

FSK All except AC9 are stimulated

PKA AC5 & AC6 are inhibited

PKC Stimulates AC1, AC2, AC3 & AC7; also AC5 by PKC-α & -ζ

Calcium

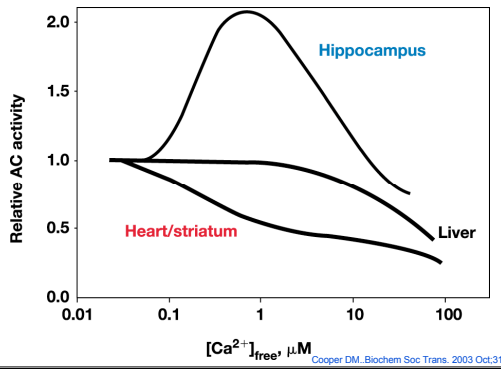
Inhibits: all cyclases at high concentration; AC5 & AC6 at micromolar; AC1 via calmodulin kinase(CaMK) IV, AC3 via CaMK II; AC9 via calcineurin

Stimulates: AC1 & AC8 via calmodulin; AC5 via PKC-α, AC2 & AC7 via PKC

No effect on AC2, AC4, and AC7

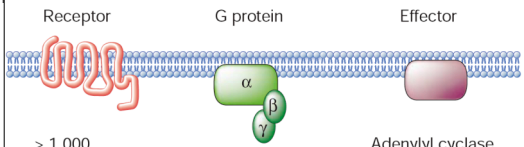
FSK, forskolin; PKA, protein kinase A; PKC, protein kinase C

Differential Cyclase (AC) Responses to Calcium



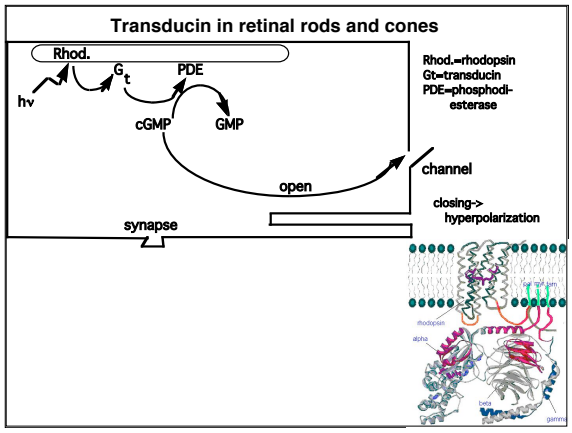
Cooper DM..Biochem Soc Trans. 2003 Oct;31(Pt 5):912-5

Many Other Effectors:



> 1,000 Adrenergic Dopaminergic Opioid Sensory – rhodopsin, olfactory, taste Glycoprotein hormones Lipids and other small molecules	15 α-subunits 6 β-subunits 12 γ-subunits	Adenylyl cyclase Phospholipases C & A_2 cGMP phosphodiesterase Potassium channels Calcium channels

Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. Nature. 2002 Jan 10;415(6868):206-12 http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v415/n6868/full/415206a_fs.html



Other classes of "G" protein

GTPase functions:

Protein synthesis (EF-tu),

Signaling (GPCR, heterotrimeric)

ER-translocation (SRP, SRP-R)

Differentiation and proliferation (ras)

Vesicular traffic (rabs)

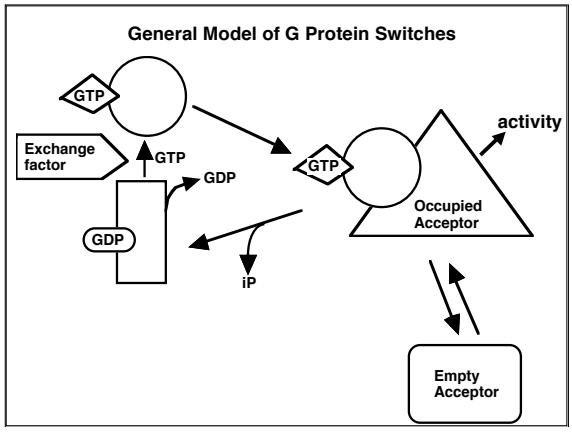
Cytoskeleton (rac and rho)

Not Identical:

Gα =350-400 amino acids

ras-like = 200 aa

EF-Tu 400-900 aa



Methods used to study G systems:

** Cholera toxin ADP-ribosylates arginine (from NAD+) Gs -, inhibits GTPase activity

** Pertussis toxin ADP-ribosylates cys near C-terminus of Gi [whooping cough] inactivates, blocks Gi interaction with receptors

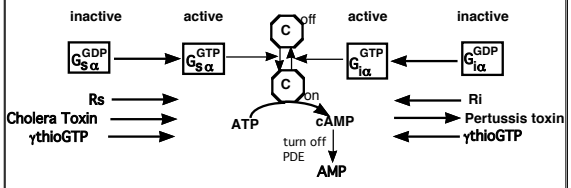
ARF: ADP-ribosylating factor required for toxin activities,

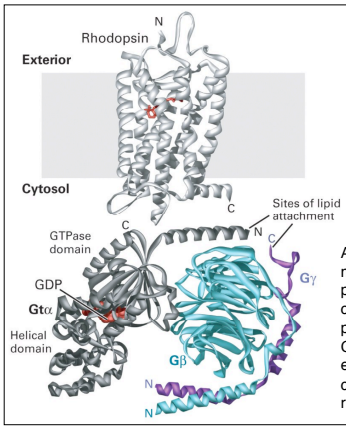
** Forskolin directly activates cyclase

** γthio-GTP, non-hydrolyzable analogue, activates

** F_4Al^-, with GDP mimic γ- phosphate group of GTP-trimeric

Methods used to study G systems:





Ligand binding sites vary with ligand structure and subclass of receptor:
- TM core
- TM core & loops
- Loops & N-term
- Protease activated

Agonist binding-> TM3/TM6 movement-> uncovers G-protein binding sites in cytoplasmic loops -> binds G-protein (GDP form) -> releases GDP (in absence of GTP, empty G protein forms stable complex with agonist bound receptor) -> GTP binds -> etc.

REGULATION OF ACTIVITY

Desensitization: tendency of biological responses to wane over time despite continuous presence of stimulus of constant intensity.

Homologous desensitization : Receptor ligand induces the loss of that receptor's activity (but not the activity of other receptors)

Heterologous desensitization: Stimulation of one receptor induces the loss of a different receptor's activity.

Phosphorylation on 5-6 loop and C-terminus

Activity Switching

Receptor Homo- and Heterodimerization

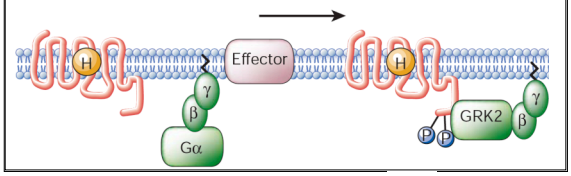
REGULATION OF ACTIVITY

G Protein Coupled Receptor Kinases (GRKs)

Identified first for Rhodopsin (GRK1), then βARK (β adrenergic receptor kinase, GRK2)

Only 7 mammalian GRKs (GRKs 2, 3, 5 & 6 ubiquitous) for ~1000 GPCRs!

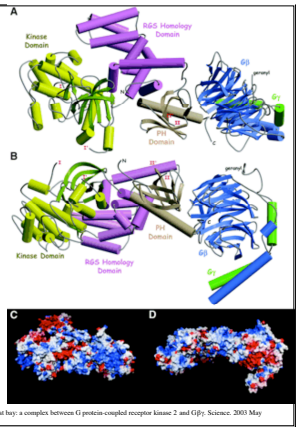
Phosphorylate Ser/Thr of ligand-bound receptor



GRK2 has three domains:

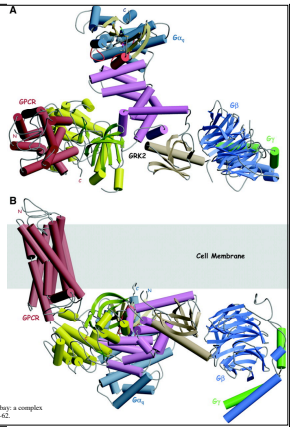
1. a predominantly N-terminal RGS (regulator of G protein signaling) homology (RH) domain
2. a central protein kinase domain, &
3. a C-terminal pleckstrin homology (PH) domain

The RH and kinase domains are common to all GRKs, whereas the PH domain is unique to GRK2 and GRK3. Other GRKs have different domains at their C termini that are similarly involved in membrane targeting.



From: Lodowski DT, Pitcher JA, Capel WD, Lefkowitz RJ, Tesmer JJ. Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and Gβγ. Science. 2003 May 23;300(5623):1256-62.

GPCR, GRK & G protein Complex



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REGULATION OF ACTIVITY

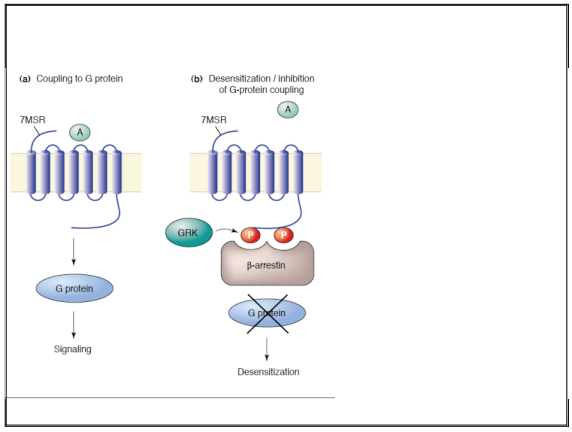
Regulators of G-protein signaling (RGS)

- Identified in yeast and worms first
- GTPase activating proteins (GAPs) for Gα's
- ~100-fold increase in GTPase rate (lower activation energy)
- controled by 14-3-3 proteins (P-ser/thr binding proteins)

REGULATION OF ACTIVITY

Arrestins

- Identified first for Rhodopsin (retinal arrestin), then β Adrenergic Receptor (βarrestin-1, βarrestin-2)
- Only 3 mammalian arrestins for ~1000 GPCRs! (retinal arrestin, βarrestin-1, βarrestin-2)
- Bind to Phosphorylated GPCRs
- Mediate multiple activities



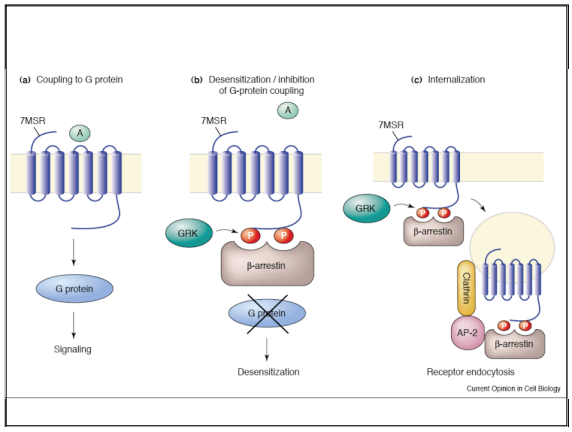
REGULATION OF ACTIVITY

β Arrestins

Block Receptor/G Protein Interaction (desensitize)

Agonist-dependent Adaptor proteins:

Endocytosis (Clathrin, AP2, ARF6, ARNO, NSF), arrestin ubiquitination for endocytosis



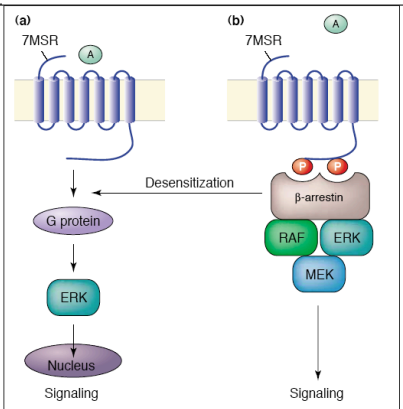
REGULATION OF ACTIVITY

β Arrestins

Block Receptor/G Protein Interaction (desensitize)

Agonist-dependent Adaptor proteins:

Endocytosis (Clathrin, AP2, ARF6, ARNO, NSF), arrestin ubiquitination for endocytosis
Multiple kinase pathways (MAPK, src, RTKs), scaffold function for assembly of complexes (src, MAPK, P38, JNK, AKT, etc.)



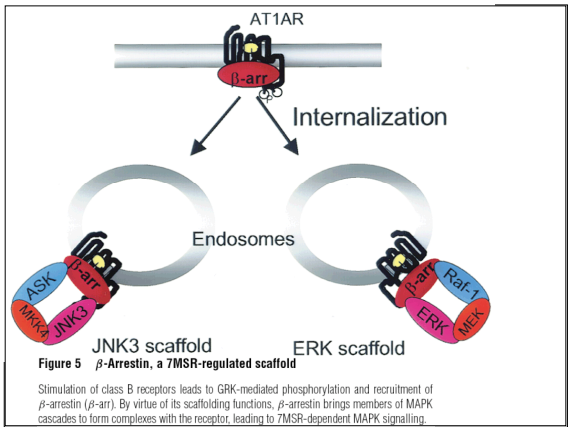


Figure 5 **β-Arrestin, a 7MSR-regulated scaffold**

Stimulation of class B receptors leads to GRK-mediated phosphorylation and recruitment of β-arrestin (β-arr). By virtue of its scaffolding functions, β-arrestin brings members of MAPK cascades to form complexes with the receptor, leading to 7MSR-dependent MAPK signalling.

REGULATION OF ACTIVITY

β Arrestins

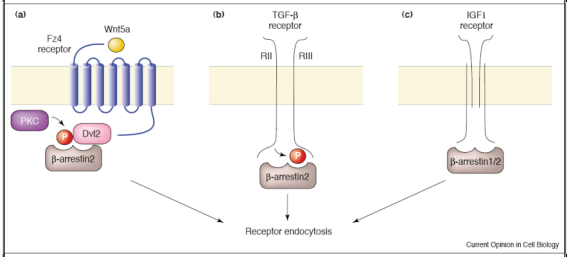
Block Receptor/G Protein Interaction (desensitize)

Agonist-dependent Adaptor proteins:

Endocytosis (Clathrin, AP2, ARF6, ARNO, NSF), arrestin ubiquitination for endocytosis
Multiple kinase pathways (MAPK, src, RTKs), scaffold function for assembly of complexes

Activate cAMP Phosphodiesterase (desensitization)

Non-GPCR receptor/channel modulation

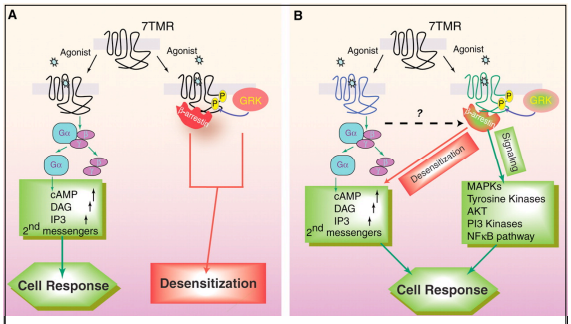


Binding protein	β-Arrestin isoform	Functional consequence
	Trafficking proteins	
Clathrin	β-Arrestin 1, 2	Endocytosis
AP2	β-Arrestin 1, 2	Endocytosis
NSF	β-Arrestin 1	Endocytosis; recycling
	Small G/GEFs	
ARF6	β-Arrestin 2≫1	Endocytosis
ARNO	β-Arrestin 2	Endocytosis
Ral-GDS	β-Arrestin 1, 2	Ral-mediated cytoskeletal changes
RhoA	β-Arrestin 1	Angiotensin II-dependent stress fiber formation
	Signaling proteins	
MAPK cascade components		
ASK1	β-Arrestin 1, 2	JNK3 and p38 activation
c-Raf-1	β-Arrestin 1, 2	ERK activation
JNK3	β-Arrestin 2≫1	Stabilization of pJNK on endosomes
ERK2	β-Arrestin 1, 2	Stabilization of pERK on endosomes
Nonreceptor tyrosine kinases		
c-Src	β-Arrestin 1, 2	Endocytosis, ERK activation
Yes	β-Arrestin 1	Gαq activation and GLUT4 transport
Hck	β-Arrestin 1	Exocytosis of granules in neutrophils
Fgr	β-Arrestin 1	Exocytosis of granules in neutrophils
Others		
Mdm2	β-Arrestin 1, 2	Ubiquitination, endocytosis
IκBα	β-Arrestin 1, 2	Stabilization of IκBα upon β2AR and TNFR stimulation
PDE4D family	β-Arrestin 1, 2	cAMP degradation
Dishevelled	β-Arrestin 1	Increase in TCF/LEF transcription
Dishevelled	β-Arrestin 2	Endocytosis of Frizzled4
PP2A	β-Arrestin 1	Ser^{*12} dephosphorylation

Table 1. A list of β-arrestin–interacting proteins.

ARF, ADP ribosylation factor; ARNO, ARF nucleotide exchange factor; IkBa, inhibitor of nuclear factor kB; PDE4D, phosphodiesterase 4D; PP2A, protein phosphatase 2A; Ral, members of the Ras superfamily of small guanosine triphosphatases (GTPases); Ral-GDS, Ral guanosine diphosphate (GDP) dissociation stimulator; RhoA, a small GTPase; small G/GEFs, small GTPase and guanine nucleotide exchange factors.

Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science. 2005 Apr 22;308(5721):512-7.



Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science. 2005 Apr 22;308(5721):512-7.

REGULATION OF ACTIVITY

Desensitization: tendency of biological responses to wane over time despite continuous presence of stimulus of constant intensity.

Homologous desensitization : β-adrenergic receptor kinase (BARK) moves from cytoplasm to complex with Gβγ, binding site overlaps with C-terminal PH domain . BARK phosphorylates receptor and lowers activity, after phosphorylation, β -arrestin binds and really drops activity. BARK member of the GRKs

Heterologous desensitization: stimulation of other receptors, PKA plays a role, PKA is about 6x slower than BARK, arrestin independent.

Phosphorylation on 5-6 loop and C-terminus

Activity Switching

REGULATION OF ACTIVITY

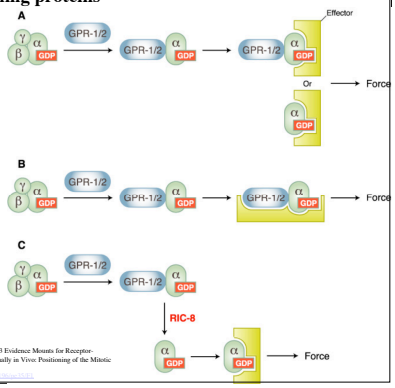
Activators of G protein signaling (AGS) or G protein regulatory (GPR) motif containing proteins

Receptor-independent activation of heterotrimer G proteins

Lanier SM. AGS proteins, GPR motifs and the signals processed by heterotrimeric G proteins Biol Cell. 2004 Jun;96(5):369-72

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VRK-4CDJ867-1&_user=501045&_coverDate=06%2F30%2F2004&_alid=202402799&_rdoc=1&_fmt=full&_orig=search&_qd=1&_cdi=6237&_sort=d&_docanchor=&view=c&_acct=C000022659&_version=1&_urlVersion=0&_userid=501045&md5=d1554586a965bcf884bafaf106b68aca&artImgPref=F

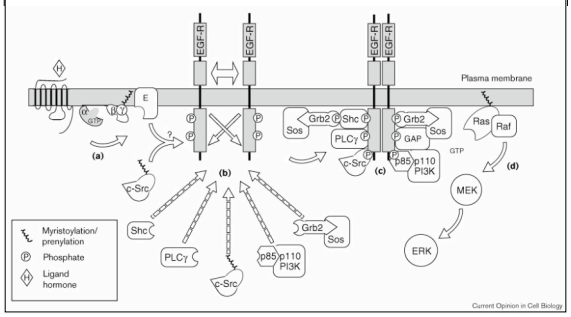
Activators of G protein signaling (AGS) or G protein regulatory (GPR) motif containing proteins



Sci. STKE, Vol. 2003, Issue 196, pp. pc35, 19 August 2003 Evidence Mounts for Receptor-Independent Activation of Heterotrimeric G Proteins Normally in Vivo: Positioning of the Mitotic Spindle in C. Elegans David R. Manning

Molecular Cross-Talk in Signaling of GPCR with Tyrosine Kinases

Luttrell LM, Daaka Y, Lefkowitz RJ. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol. 11(2):177-183. (1999)



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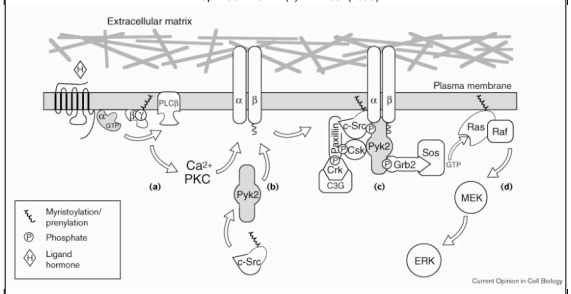
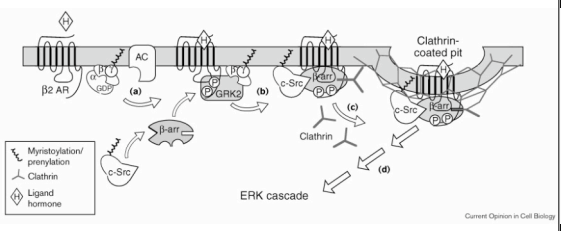


ERK activation mediated by Pyk2 recruitment to focal adhesions

Molecular Cross-Talk in Signaling of GPCR with Tyrosine Kinases

Luttrell LM, Daaka Y, Lefkowitz RJ. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. Curr Opin Cell Biol. 11(2):177-183. (1999)



β-arrestin-dependent formation of a Src signaling complex with β2 adrenergic receptor

Diseases and G Proteins: Both loss of function and gain of function mutations

- **Pseudohypoparathyroidism type Ia (PHP-Ia) has 30-50% Gs activity, appears to be autosomal-dominant, primary defect not always certain**
- **Cholera (salt secretion) and whooping cough**
- **Lithium at therapeutic concentrations (0.6 mM) blocks coupling of muscarinic and β- adrenergic receptors to G proteins, may be basis of some of Li^+ effects in manic depression, although PIP_2 effect may be far more important**
- **Growth hormone-secreting pituitary tumors with somatic mutations in Gs, Gαs activated by mutation inhibiting GTPase, same defect in 3/11 adrenal cortex and 3/10 ovarian endocrine tumors in Gi2 chain and in autonomously functioning thyroid adenoma**

Diseases and G Proteins: Both loss of function and gain of function mutations

-Acromegaly-hypersecretion of growth hormone by somatotrophs in pituitary, very small tumors, two classes of tumors, one is a $G_{s\alpha}$ defect -> Growth hormone constitutively secreted, adenylate cyclase only needs GTP, GHRH independent cAMP, Gln 227 and Arg 201 mutations

--Chemokine receptor CCR5, co-receptor fo HIV, mutation prevents binding of HIV to target cells; homozygotes protected from HIV

--Combined testotoxicosis and pseudohypoparathyroidism type Ia (PHP-Ia) - rare

Iiri T, Herzmark P, Nakamoto JM, van Dop C, Bourne HR Rapid GDP release from Gs alpha in patients with gain and loss of endocrine function. *Nature* 1994 Sep 8;371(6493):164-168

Other Second Message Systems:

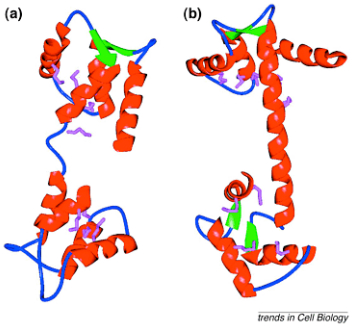
Ca^{++}: intracellular [Ca^{2+}] usually 10^{-7}->10^{-5}M

Transient increases (sparks, waves)-quickly pumped out or binding proteins sequester. Some effectors bind Ca^{++} directly (PK-C). Many use intermediates: troponin C (muscle), S100 family, recoverin, frequenin, and best understood…

Calmodulin (17kD,4 binding sites,
K_d for 2 carboxy-term= 10^{-7} M, for 2 N-term=$2.4x10^{-6}$)

Ca^{2+} Binding exposes hydrophobic side chains:

Structure of Calmodulin (CaM):



a) Ca^{2+} -free (apoCaM)
b) 4xCa^{2+} -CaM

Ca^{++} enters cytosol from extracellular fluid or from ER

Source, frequency and amplitude of spikes in conc. influence effects of the signal!

Nature Reviews Molecular Cell Biology 1: 11-21 (2000)

THE VERSATILITY AND UNIVERSALITY OF CALCIUM SIGNALLING

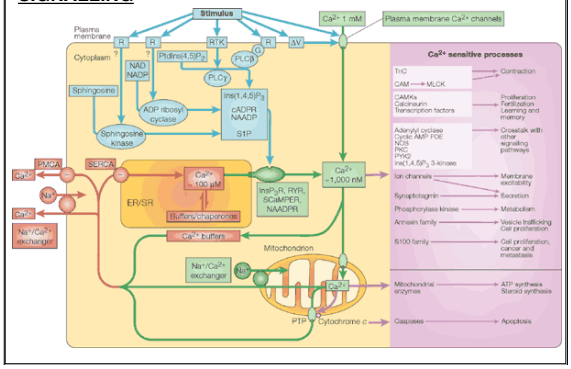
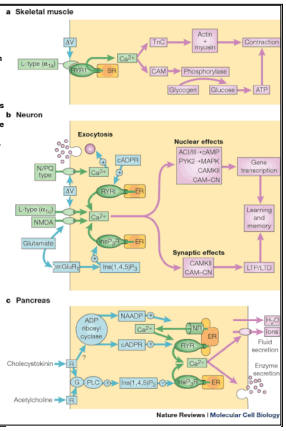


Figure 4 | Application of the Ca2+ signalling toolkit to regulate different cellular processes.

a | In skeletal muscle, an L-type Ca2+ channel (1S) senses membrane depolarization (V) and undergoes a conformational change that is transmitted to the ryanodine receptor 1 (RYR1) (Fig. 3b). Ca2+ released from the sarcoplasmic reticulum (SR) interacts with two sensors, troponin C (TnC), which triggers contraction, and calmodulin (CAM), which activates glycogen metabolism to synthesize ATP.

b | Neurons have several Ca2+-sensitive processes located in different regions. Membrane depolarization (V) is sensed by N- or P/Q-type channels at the synaptic endings to produce a localized pulse of Ca2+ that triggers exocytosis. In the cell body and dendrites, L-type channels sense the same depolarization and induce the entry of Ca 2+ which has a number of targets: adenylyl cyclase I or III (AC I/III) leading to cyclic AMP production, proline-rich tyrosine kinase (PYK2), mitogen-activated protein kinase (MAPK), Ca2+/calmodulin-dependent protein kinase II (CAMKII) and calmodulin–calcineurin (CAM–CN). Some of these targets induce gene transcription. The neurotransmitter glutamate can also generate Ca2+ signals either by activating receptor-operated channels such as NMDA (N-methyl-D-aspartate) receptors, or by stimulating the metabotropic glutamate receptor mGluR1 to produce inositol-1,4,5-trisphosphate (Ins(1,4,5)P3) to mobilize internal Ca2+ from the endoplasmic reticulum (ER). These glutamate-induced Ca2+ signals are localized to synaptic endings, where they contribute to processes such as long-term potentiation (LTP) and long-term depression (LTD), which have been implicated in learning and memory.

c | The exocrine pancreas uses two signalling systems regulated by separate receptors. Acetylcholine uses Ins(1,4,5)P3 to release internal Ca2+. As well as stimulating Ins(1,4,5)P3 formation, cholycystokinin also acts through both cyclic ADP ribose (cADPR) and nicotinic acid dinucleotide phosphate (NAADP). The latter seems to act by releasing a small amount of trigger Ca2+ through the NAADP receptor (NR) that then acts together with cADPR to release further Ca2+ through RYRs.



Nature Reviews | Molecular Cell Biology

Ca2+ nexus — crosstalk between signalling pathways

The Ca2+ signalling interacts with many other pathways. The interactions are reciprocal, in that information flows to and from the pathways. Each set of interactions has to be treated separately:

Ca2+–cyclic AMP interactions (1)

The function of the cyclic AMP and Ca2+ signalling systems are intimately linked. Some of the adenylyl cyclase isomers are activated by Ca2+, others are inhibited. Ca2+ can also stimulate some cAMP phosphodiesterases (cAMP PDE). Changes in the level of cAMP can feed back to influence the level of Ca2+ by acting on both Ca2+ channels and pumps. In cardiac and skeletal muscle, the activity of the L-type Ca2+ channel is enhanced by cAMP.

Ca2+–NO interactions (2)

An important function of Ca2+ is to activate nitric oxide (NO) synthase to generate NO, which functions as a local hormone to regulate the activity of neighbouring cells. The NO activates guanylyl cyclase to produce cyclic GMP, which feeds back to influence the activity of Ca2+ channels and pumps. For example, smooth muscle cells relax when cGMP phosphorylates an inositol-1,4,5-trisphosphate receptor (InsP3R)-associated cGMP kinase substrate that reduces inositol-1,4,5-trisphosphate (Ins(1,4,5)P 3)-induced Ca2+ release108.

Ca2+–phosphatidylinositol-3-OH kinase interaction (3)

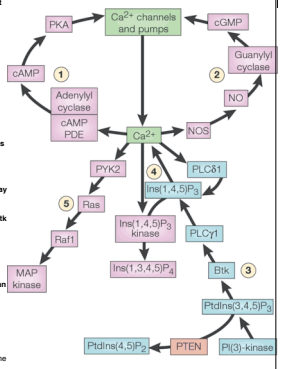
The ubiquitous phosphatidylinositol-3-OH-kinase (PI(3)K) signalling pathway has many functions in cells, which are mediated by the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3). One function of PtdIns(3,4,5)P3 is to activate the non-receptor tyrosine kinase Btk that then phosphorylates and activates phospholipase C1 (PLC1)61. The tumour suppressor PTEN, a 3-phosphatase that lowers the level of PtdIns(3,4,5)P3, reduces both the level of Ins(1,4,5)P3 and the influx of external Ca2+ (Ref. 109).

Ca2+ feedback interactions (4)

There are numerous feedback interactions within the Ca2+ signalling pathway whereby Ca2+ can modulate its own activity. For example, Ca2+ can activate phospholipase C1 (PLC1) to increase the level of Ins(1,4,5)P3. Conversely, it can lower the level of this second messenger by stimulating the Ins(1,4,5)P3 kinase to produce Ins(1,3,4,5)P4. Finally, Ca2+ can exert profound effects on the Ca2+ channels and pumps.

Ca2+–mitogen-activated protein kinase interaction (5)

Ca2+ can interact with the mitogen-activated protein kinase (MAPK) signalling pathway by activating a proline-rich tyrosine kinase 2 (PYK2), which then acts through the small GTPase Ras to induce the MAPK cascade110. For example, the growth of smooth muscle cells may depend on the Ca2+-dependent activation of the MAPK pathway111. A more indirect method may depend on Ca2+ stimulating a metalloproteinase to release epidermal growth factor (EGF) from a precursor, as seems to occur in prostate carcinoma cells112.



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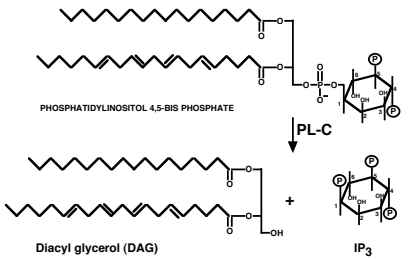
The Phosphatidylinositol 4,5 bisphosphate (PIP_2) system:

PI->PI(4)P->PI(4,5)P_2



Diacyl glycerol (DAG) **IP_3**

Phospholipase-C: PL-C
2-8% of membrane lipid is PI, 10-20% of PI are poly phosphorylated

Nomenclature: Ins(x,y,z)P_3 x,y,z are the positions of Phosphate; Ins(4)P - D-myo-inositol 4-monophosphate; Ins(1,4,5)P3 - D-myo-inositol 1,4,5-bisphosphate; Ptdins - phosphatidylinositol

Experimental findings which have lead to the current model:

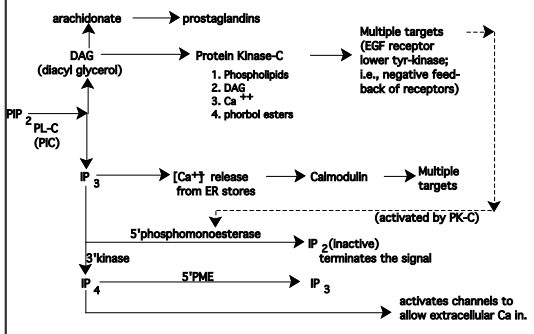
1. In 1950's cholinergic stimulation of enzyme secretion by pigeon pancreas slices increased incorporation of [^{32}P] into "RNA fraction", which was later shown to be phospholipids, muscarinic antagonist atropine blocked.

2. In 1958 shown that incorporation was into PI and [^{3}H] myoinositol incorporation was stimulated.

3. In 1981, polyphosphoinositide breakdown was shown to be a very early event after hormone treatment;

4. Use of lithium to inhibit hydrolysis of IP_1 to myol + P provided more sensitive assay for accumulation of phosphorylated inositols,

5. Careful kinetics showed IP_3 release with PIP_2 loss; finally, permeabilized cell and cell injection systems shown that IP_3 (micromolar) releases Ca from nonmitochondrial store in permeabilized pancreatic acinar cells and DAG can both act as messages.



Methods:

Systems studied: add IP_3 to permeabilized cells or inject

nonhydrolyzable analogue: inositol 1,4,5-trisphosphorothioate

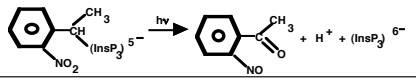
Ca-sensitive dyes for temporospacial monitoring

Lithium inhibits IP phosphatase (IP->myoinositol + P)

- manic depression and development (teratogenic, dorsal-ventral axis); 4-phosphatase enriched in the brain

Ca via ionophors (e.g.A23187) or PK-C via phorbol esters

Photolabile "caged" precursor (2-nitrophenylethyl phosphate esters)



Protein Kinase C (PKC)

single chain of **77 kD**

***Discovered by Nishizuka and colleagues 1977**

***cytoplasmic-inactive // translocated to plasma membrane on binding DAG-active**

***DAG induces translocation to membrane and increases affinity for Ca^{++} to normal physiologic levels, DAG binds stoichiometrically and steriospecifically (sn12, not sn2,3)**

***Requires acidic phospholipid (probably PS), 4-10 per enzyme**

***multiple isozymes -11 identified by cDNA screening,**

***phorbol esters and other tumor promoters bind at diglyceride site and activate PK-C, more potent than DAG and not readily metabolized**

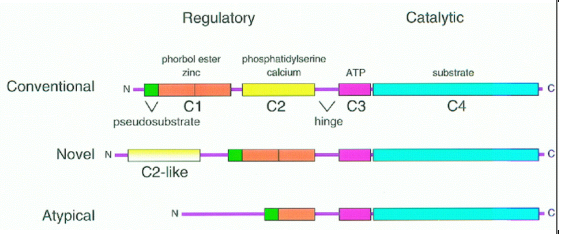
nhibitors: cationic amphipathic, e.g., chlorpromazine, trifluoperazine and sphingosine

Multiple Isoforms of PL-C: 56.6 kD (PLC-α),138.2 kD (PLC-β), 148.4 kD (PLC-γ) 85.8 kD (PLC-δ)

Structure:

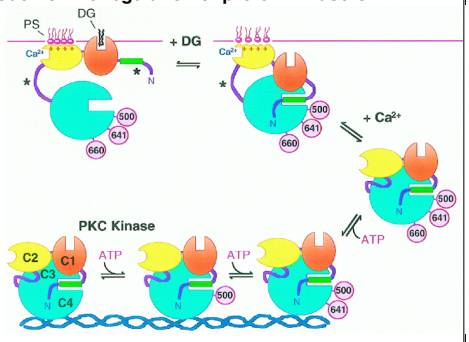
2 major domains (regulatory/kinase) with subdomains:

Regulatory domain- N-terminal, "zinc fingers"



Alexandra C Newton: Regulation of protein kinase C *Current Opinion in Cell Biology* 1997 9 : 161-167.

Model for the regulation of protein kinase C



Alexandra C Newton: Regulation of protein kinase C *Current Opinion in Cell Biology* 1997 9 : 161-167.

**pseudosubstrate site in aa19-36 (R^{19}-F-A-R-K-G-A^{25}-L-R-G-K-N-V-H-D-V-K-N^{36}) peptide is antagonist (Ki=147 nM, inhibits, substitution of A25 for Ser gives good substrate)

**removed by Ca-dependent protease (Calpain I) in presence of TPA, DAG etc. -> 51 kD (constitutively active, unstable) and 26 kD (regulatory) fragments

experimental trick: treat cells with high levels of TPA, short term increase in PK-C activity, but overnight functionally PK-C depleted

NOTE COMMON REGULATORY MECHANISM:

Built-in inhibitor is released on activation

PK-C (covalently associated inhibitory domain)-pseudosubstrate

cAMP-dependent PK (regulatory subunits released by cAMP binding) also inhibitor protein which contains pseudosubstrate R-R-N-A-I; substrate is R-R-X-Ser(P)-X

cGMP-PDE of ROS (γ inhibitory subunit released by T_α of transducin)

myosin light chain kinase (substrate-like inhibitory tail, calmodulin binds to release)

Ca/Calmodulin-dependent protein kinase: hexamer, internal pseudosubstrate inhibits, Ca/calmodulin binding allows autophosphorylation and Ca/Calmodulin independence

end

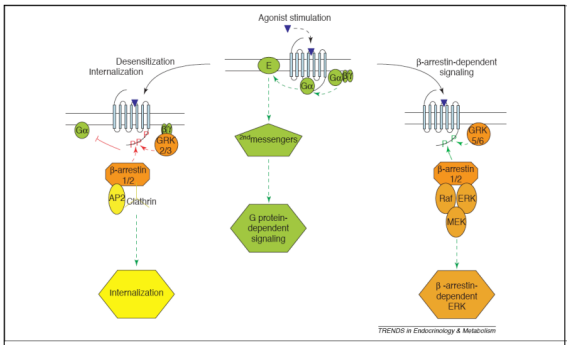


Figure 1. Functional specialization of the different GRKs: a model. Upon agonist stimulation, GRK2 and -3 are recruited to the plasma membrane by interacting with Gβγ subunits. They have a predominant role in receptor phosphorylation, β-arrestin recruitment to the activated receptor, desensitization and internalization. GRK5 and -6 are constitutively associated with the plasma membrane. They are both required for β-arrestin-dependent ERK activation, although the locus of their action might be at the receptor or downstream. In some systems, GRK5 and -6 can also mediate desensitization and internalization. Green-dotted arrows: activation; red-dotted arrows and red lines: inhibition. Abbreviation: E, second messenger-generating enzyme.

Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol Metab. 2006 May-Jun;17(4):159-65.

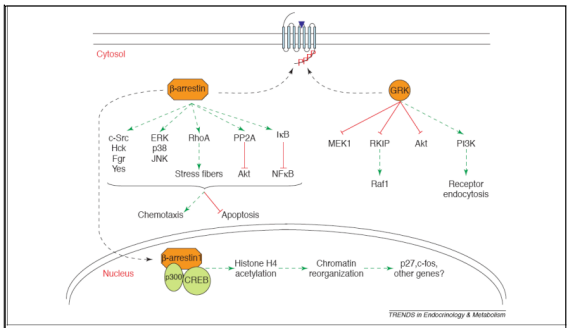


Figure 2. An overview of the current GRK- and β-arrestin-mediated signaling functions and biological activities. Upon agonist stimulation of a 7TMR, β-arrestins and GRKs are recruited and activated. They subsequently activate (green dotted arrows) or inhibit (red plain lines) various signaling pathways. β-arrestin signaling is important for chemotaxis and antiapoptosis. In addition, β-arrestin 1 can translocate to the nucleus and regulate the expression of various genes through reorganization of chromatin. Abbreviations: c-Src, protooncogene SRC, Rous sarcoma; Hck, non-receptor tyrosine protein kinase Hck; Fgr, Gardner-Rasheed feline sarcoma viral oncogene homolog; Yes, non-receptor protein tyrosine kinase Yes; ERK, extracellular signal regulated kinase; p38, p38 mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; RhoA, Ras homolog gene family member A; PP2A, protein phosphatase 2A; Akt, protein kinase B; NFκB, nuclear factor kappa B, IκB, nuclear factor kappa B inhibitor; MEK1, mitogen activated protein kinase kinase 1; RKIP, Raf kinase inhibitor protein; Raf1, mitogen activated protein kinase kinase kinase 1; PI3K, phosphoinositide 3 kinase. Green-dotted arrows: activation; black-dotted arrows: translocation; red lines: inhibition.

Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol Metab. 2006 May-Jun;17(4):159-65.

Box 1. Impact of β-arrestin-dependent signaling on pharmacological screening

Pharmacological agents acting via 7TMRs have traditionally been discovered through the screening of numerous chemical structures in biological systems. Clearly, the type of receptor screen employed to detect biologically active molecules will greatly define the types of molecules detected. For many years, these functional high-throughput assays have employed second messenger generation, thus G protein activation, as the read-out. The drugs detected by such screens have been agonists, partial agonists, antagonists or inverse agonists for G protein activation. However, the recent demonstration that specific ligands are able to activate β-arrestin-dependent, G protein-independent signaling implies that multiple discrete 'active' receptor conformations coexist [48,50,54,68]. It is now possible to develop assays to screen compound libraries systematically using β-arrestin recruitment (e.g. confocal microscopy, fluorescence resonance energy transfer- or bioluminescence resonance energy transfer-based assays) or β-arrestin-mediated signaling (e.g. activation of a specific gene reporter) as read-outs for 7TMR activation. This new generation of high throughput screens will potentially lead to the identification of new pathway-selective drugs that might have valuable therapeutic properties.

Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol Metab. 2006 May-Jun;17(4):159-65.