Signaling via G protein-coupled receptors
(GPCRs)
McGinnis Nov. 15, 06 BGGN 220
Discovery of G protein coupled (7-transmembrane) receptor pathways

Martin Rodbell (NL) showed in the 1960s that GTP was involved in cell signaling.

Al Gilman (NL) discovered the proteins that interacted with GTP to initiate signalling cascades within the cell.

Hargrave et al. and Argos in obscure journal in 1983 predict bovine rhodopsin has seven transmembrane helical structure.

Bob Lefkowitz, Duke, and colleagues cloned β-2 adrenergic receptor in 1986, and many others by sequence homology, e.g. serotonin receptor, and noted sequence similarity to opsins in 7 predicted transmembrane domains.
GPCRs Affect Second Messenger Production Through the Action of G-proteins

~ 70% of all prescription drugs act on GPCRs, and their constitutive activation can lead to defects both profound and sometimes bizarre, e.g. premature puberty in males (i.e. a few years old) due to lutenizing hormone LH receptor activation
Components of G protein-coupled pathways

- Receptors - binds ligand
- G proteins - comprised of three subunits, α, β, γ.
  - The α subunit binds guanine nucleotides (thus G proteins), which regulates a change in structure.
  - The βγ subunits form a complex and do not dissociate.
- Downstream effectors - both G α and G βγ subunits activate effectors.
Proposed arrangement of serpentine receptor helices

Extracellular loops: Polypeptide hormone binding

Phosphorylation mediated adaptation

View from above the membrane

Helix lined pocket: biogenic amine and retinal binding

Structural Characteristics of GPCRs
GPCRs Affect Second Messenger Production Through the Action of G-proteins

- > 1000 GPCRs
- ~ 20 Different G-proteins
- ~ 12 Different Effector Enzymes

Several Different Hormone Receptor Complexes can activate a given G-protein, and much of the specificity of GPCR signaling is dependent on the expression pattern of the receptors, e.g. gut or cerebral cortex. As the ligands circulate rather freely, albeit many have short half lives.
Heterotrimeric G-protein Subunits

Farnesyl (15 C isoprenoid)  Myristoyl group (CH$_3$)-(CH$_2$)$_{14}$-C=O)

20 genes - $G_\alpha$ subunits
5 genes - $G_\beta$ subunits
10 genes - $G_\gamma$ subunits
# G-protein families

<table>
<thead>
<tr>
<th>G-protein family</th>
<th>Gα Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gs</td>
<td>Stimulate Adenylate cyclase</td>
</tr>
<tr>
<td>Gi</td>
<td>Inhibit Adenylate cyclase</td>
</tr>
<tr>
<td>Gq</td>
<td>Stimulate Phospholipase Cβ</td>
</tr>
<tr>
<td>G₁₂</td>
<td>Stimulate Ion Channels</td>
</tr>
</tbody>
</table>
GTPases are molecular switches

- GTPase include EF-Tu, $G_{\alpha}$ subunits, small G proteins such as Ras, Rac, Ran, etc.
- The proteins bind GTP in the basal or non-activated state and GTP in the activated state.
- The binding of GTP results in a conformational change allowing the protein to bind to downstream proteins- effectors.
- All have an intrinsic GTPase activity. The hydrolysis of GTP results in converting the protein to the basal, inactive GDP-bound state.
- The turnover rate for Ras and $G_{\alpha}i$ are $\sim 0.03$ and 3 molecules/min, respectively.
- These rates are stimulated 100-1000-fold by GAPs (GTPase activating proteins- Ras/Rac) and RGS (regulators of G protein signaling- $G_{\alpha}$ subunits).
G-protein Signal Transducers: Basic Cycle

REGULATORS
- **GEFs**: Guanine nucleotide exchange factors
- **GDIs**: Guanine nucleotide dissociation inhibitors
- **GAPs**: GTPase activating proteins
- **GIPs**: GTPase inhibiting proteins
Heterotrimeric G-protein GTPase Cycle

GEF Activity: Stimulates GDP Exchange

Ligand-Receptor Complex

GDP Exchange

Kd-GDP

Active

GTP

Effector Enzyme

Inactive

kcat-GTP

GAP Activity

Pi

RGS
Seven-helix bundle of receptors as viewed from the cytoplasm

**Inactive State**
Salt bridge between TM 7 and 3

**Postulated Active State**
Salt bridge broken
TMs 6 & 7 lean out
=> cleft opens in the middle of the bundle

Based on 2-D crystal structures for rhodopsin
The classes (by sequence homology and ligand specificity) in the gigantic GPCR superfamily of 7 transmembrane receptors (about 5-10% of all genes in animal genomes encode GPCRs).
The actual scale of GPCRs and their heterotrimeric G-protein signaling complex

What is missing from this diagram of the structures?
Adaptation of signaling-turning off the response

1. Removal or destruction of ligand- internalization of the receptors, destruction of ligand, and return of receptors to the membrane.

2. Hydrolysis of GTP by Gα subunits. This also allows Gβγ subunits to rebind the Gα subunit, which reduces basal GDP/GTP exchange.

3. RGS, Regulators of G protein Signaling, function to stimulate the intrinsic GTPase activity of Gα subunits. Some effectors function as RGS proteins. Some RGS proteins are Gα protein specific. For other G proteins (e.g. Ras, Rac), proteins with similar functions are called GAPs- GTPase activating proteins.
GRKs- G protein-coupled Receptor Kinases
(phosphorylation of ligand-bound receptors)

GRKs, G protein receptor kinases

- RGS homol. domain
- Kinase domain
- RGS homol. domain
- binds to and targets the protein to Gα subunit
domain
- Variant domain
- Membrane targeting domain

Targeting domains- some are constitutively bound, some bind in response to signaling:
- GRK1- prenylation (farnesyl)
- GRK2, 3- Gβγ-interacting and a PH domain (binds phospholipids)
- GRK4, 6- palmitolation
- GRK5- novel, phospholipid binding

Figure 1: Domain architecture of G protein-coupled receptor kinases (GRKs). The sequences of the six known mammalian GRKs are represented schematically. The solid areas are regions of invariant amino acid sequence. The bifurcation in the C-terminal domain represents divergence in sequence between the various GRKs, where rhodopsin kinase (RK) is farnesylated, β-adrenergic receptor kinase (βARK) and βARK2 contain a βγ binding domain, GRK4 and GRK6 are palmitoylated, and GRK5 contains a basic phospholipid binding domain.
Figure 2 | Seven-transmembrane (7TM)-receptor trafficking. Activation of 7TM receptors by an agonist leads to the dissociation of $\alpha$ and $\beta\gamma$ subunits. The free $\beta\gamma$ dimers recruit G-protein-receptor kinases (such as GRK2 or GRK3) to the receptor, where they specifically phosphorylate agonist-occupied receptors. This, in turn, leads to the recruitment of $\beta$-arrestin to the receptor and targets the receptor–$\beta$-arrestin complexes to clathrin-coated pits. The receptor is internalized into acidic endosomes and then either dephosphorylated and returned to the cell surface or degraded.
Binding of GTP results in a conformational change

- The GDP- and GTP-bound forms have different conformations - the switch domains (shown colored) move.
- In the GTP-bound form, the G protein can bind to a downstream effector and activate it.
- Upon GTP hydrolysis, the protein changes to the inactive (GDP-bound) state where it interacts with the exchange factor.
Both $G\alpha^{GTP}$ and $G\beta\gamma$ can activate downstream effectors
G-protein families (~18 in most mammal genomes) and their primary downstream effectors. Predominance of phospholipids and Ca ions.

<table>
<thead>
<tr>
<th>G-protein family</th>
<th>Subunit</th>
<th>Effector Enzyme</th>
<th>2nd Messenger</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gs</td>
<td>Gsα</td>
<td>Ad cyc</td>
<td>cAMP</td>
<td>Stimulate</td>
</tr>
<tr>
<td></td>
<td>Gβγ</td>
<td></td>
<td>IP₃,DAG,Ca²⁺</td>
<td>Stimulate</td>
</tr>
<tr>
<td></td>
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<td>PLCβ</td>
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<tr>
<td></td>
<td></td>
<td>Ca²⁺ channels</td>
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<td>Gβγ</td>
<td>K⁺ channels</td>
<td>K⁺</td>
<td>ΔΨ</td>
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<tr>
<td>G12</td>
<td>G12α</td>
<td>RhoGEF</td>
<td>-</td>
<td>Stimulate</td>
</tr>
</tbody>
</table>
G\(\alpha_s\) Regulation of Adenylate Cyclase Mediated Signaling

Hormones Acting Through G\(\alpha_s\):
- ACTH- Adrenocorticotropic Hormone
- MSH- Melanocyte-Stimulating Hormone
- Glucagon
- GnRH- Gonadotropin-releasing hormone
- LH- Luteinizing hormone
- FSH- follicle-stimulating hormone
- PTH- parathyroid hormone
- epinephrine (adrenaline)

PKA preferred sites (RK)(RKS)X(ST)(FLMI)(AVI)(ILMARK)
cAMP Synthesis and Degradation

ATP

\[
\begin{align*}
\text{ATP} & \quad \xrightarrow{\text{Adenylyl Cyclase}} \quad \text{cAMP} + \text{PPi} \\
\text{AMP} & \quad \xrightarrow{\text{Phosphodiesterase}} \quad \text{ATP}
\end{align*}
\]
Domain Organization of Protein Kinase A (PKA) Regulatory and Catalytic Subunits

Catalytic Subunit

Negative Regulatory Subunit

Dimerization & Docking

Pseudo-substrate

ATP binding lobe

Substrate binding lobe

cAMP binding domains
R-Subunit Pseudo-substrate Site Is a Consensus PKA Substrate Recognition Motif

Negative Regulatory Subunit

Dimerization & Docking

Pseudo-substrate

Pseudo-substrate sequence

RI
SPPPPNPVVKGRRRRG\textcolor{red}{A}I\textcolor{red}{S}A

RII
EEDLDVIPGRFD\textcolor{red}{R}RV\textcolor{red}{S}VCA

Peptide Substrate

PKA phosphorylation

LRR\textcolor{red}{A}S\textcolor{red}{L}G
cAMP Dependent Activation of PKA

Inactive Holoenzyme Tetramer:
2 C-subunits
2 R-subunits

Pseudo-substrate inhibition
Contacts required For high affinity binding

2 Active C-subunits + Dimer cAMP bound R-subunits

Conformational change Induced by cAMP Weakens R-C binding
<table>
<thead>
<tr>
<th>Protein</th>
<th>Tissue</th>
<th>Effect of Phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen synthase</td>
<td>Liver, muscle</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Phosphorylase kinase</td>
<td>Liver, muscle</td>
<td>Stimulation</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>Liver</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Hormone-sensitive lipase</td>
<td>Adipose</td>
<td>Stimulation</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>Liver</td>
<td>Inhibition</td>
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<tr>
<td>Phenylalanine hydroxylase</td>
<td>Liver</td>
<td>Stimulation</td>
</tr>
<tr>
<td>CREB transcription factor</td>
<td>All</td>
<td>Stimulation</td>
</tr>
<tr>
<td>Gs-linked receptors</td>
<td>All</td>
<td>Adaptation=Desensitization</td>
</tr>
</tbody>
</table>
The bifunctional protein Transglutaminase 2, a Lys-Gln crosslinking enzyme, can act in multiple biochemical ways to activate GPCR signaling.
Phospholipase C

- Phospholipase C (PLC) has three different subclasses- PLCβ1 (Gαq), PLCβ2 (Gβγ), and PLCγ (Receptor tyrosine kinase pathways).
- PLC hydrolyses phosphotidyl-inositol 4,5 bisphosphate (PIP$_2$) to produce two second messengers- diacylglycerol (DAG or DG) and inositol triphosphate (IP$_3$).
- PLCβ1 also functions as a RGS for Gαq$^{GTP}$. 
Phosphatidylinositol Signal Cascades

Some hormones activate a signal cascade based on the membrane lipid **phosphatidylinositol**.
Kinases sequentially catalyze transfer of $P_i$ from ATP to OH groups at positions 5 & 4 of the inositol ring, to yield phosphatidylinositol-4,5-bisphosphate (PI(4,5)P$_2$).

PIP$_2$ is cleaved by the enzyme Phospholipase C.
Cleavage of PIP$_2$, catalyzed by Phospholipase C, yields two second messengers: inositol-1,4,5-trisphosphate (IP$_3$) & diacylglycerol (DG).

Diacylglycerol, with Ca$^{++}$, activates Protein Kinase C, which catalyzes phosphorylation of several cellular proteins, altering their activity.
PKC- a primary downstream effector of PLC activation

- The two PLC-produced second messengers, one soluble (IP₃) and one lipid soluble thus remaining within the plasma membrane (DG) function to activate protein kinase C (PKC).

- IP₃ binds to IP₃ receptors in the ER to release Ca²⁺ into the intracellular stores.

- Ca²⁺ functions coordinately with DG to activate PKC.

- DG binds to the Cys-rich in PKC, leading to its translocation to the plasma membrane.

- The role of DG can be mimicked by the tumor promoter phorbol esters, (plant derived terpene tricyclic compounds) which lead to constitutive activation of PKC.

- PLCβ1 also functions as a RGS for Gαq<sub>GTP</sub>., thus providing a mechanism to down-regulate/adapt the pathway.
Gαq Regulation of PLC Mediated Signaling

Hormone

Receptor

Gq

β γ

αq

GTP

PLCβ

PI cycle

PIP2

DAG

IP3

IP3

R

PKC

C

inactive

active

Target

Ca2+

HO−

PO4−
PKC Domain Structure

A. Cysteine-Metal Fingers:
   DAG binding

Acidic Domains:
   Ca\(^{2+}\) Binding

ATP Binding

Substrate Interaction

Catalytic

Pseudo-substrate Site

Regulatory
2nd Messenger Activation of PKC

Inactive

Active

- DAG Binding Site
- Ca$^{2+}$ Binding Site

Pseudosubstrate Site

DAG

Ca$^{2+}$
Gi Regulation of Adenylyl Cyclase and PLC Mediated Signaling

Target Proteins

Receptor

Gi

αi

β

γ

GDP

αi

ATP

cAMP

Target Proteins

Ligand

PLCβ

IP3

IP3

Ca2+

Cyclic GMP

Cyclic AMP

e.g.

Norepinephrine:

α2-adrenergic receptor (presynaptic nerve terminals)
Alterations That Can Block Pathway Function

Ligand-Receptor

G-protein

a. Reduced expression
- deletion mutation
- defects in transcription
- defects in mRNA processing or stability
- defects in membrane targeting

b. Impaired activation
- aa substitutions: uncouple ligand/receptor receptor/G-protein
- alterations in adaptive processes that decrease time in active state

Background . . . . what is known about RGS proteins like yeast Sst2 and more importantly, what is unknown, and what do the authors wish to elucidate that is (or might be) novel?
Fig. 1
DEP domain requirement in Sst2
Fig. 2
DEP domain specificity for a yeast signaling pathway
Fig 3.
Rescue of Sst2 mutant by more Ste2
Fig 4.
Sst2 binds to . . .
Sst2 colocalizes in yeast cells with...
Fig 6.
Sst2 colocalizes, or is it associates or even binds to . . . . in a certain unmodified state . . .
Fig. 7.
The conceptual cartoon model.

how well do the results support
the authors claims of
interaction, specificity,
and novelty
(and relevance to any GPCR
pathway outside yeast)?