Johns Hopkins Engineering

Molecular Biology

GPCRs and Second Messengers

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Outline

- Receptor categories
- GPCRs – G-protein coupled receptors
- Second messengers

Categories of receptors

- Receptors can be classified into several basic categories

  - Ligand-gated channels (e.g. ion channels that allow neurotransmitters to pass through)

  - Plasma membrane receptors of two types

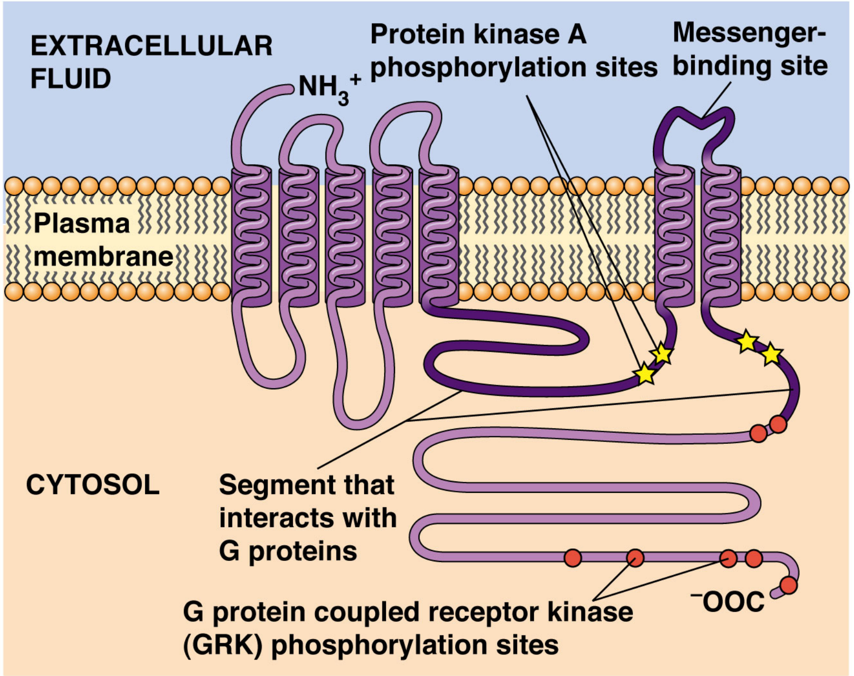
    - **Those linked to G proteins**
    - Those linked to protein kinases

G Protein-Linked Receptors

- The **G protein-linked receptor family** is so named because ligand binding causes a change in receptor conformation that activates a particular **G protein (**G protein = *guanine-nucleotide binding protein; a.k.a. GDP and GTP)*
- The G protein then binds a target protein, such as an enzyme or channel protein, thus altering the target's activity
- **Many Seven-Transmembrane Receptors Act via G Proteins**
- A class of G-protein-coupled receptors (GPCRs) of great clinical importance is the *opioid receptors*, to which narcotic drugs such as morphine bind

The Structure and Regulation of G Protein-Linked Receptors

- G protein-linked receptors all have a similar structure but quite different amino acid sequences
- The receptor forms seven transmembrane α helices connected by alternating cytosolic or extracellular loops
- The extracellular portion of each receptor has a unique messenger-binding site



The Structure, Activation, and Inactivation of G Proteins

- G proteins act like molecular switches whose on and off states depend on whether they are bound to GTP (guanosine triphosphate) or GDP (guanosine diphosphate)
- There are *large heterotrimeric G proteins (containing three subunits)* and *small monomeric G proteins*
- The heterotrimeric G proteins mediate signal transduction through G protein-linked receptors and have G_{α}, G_{β}, and G_{γ} subunits

G protein Structure & Function

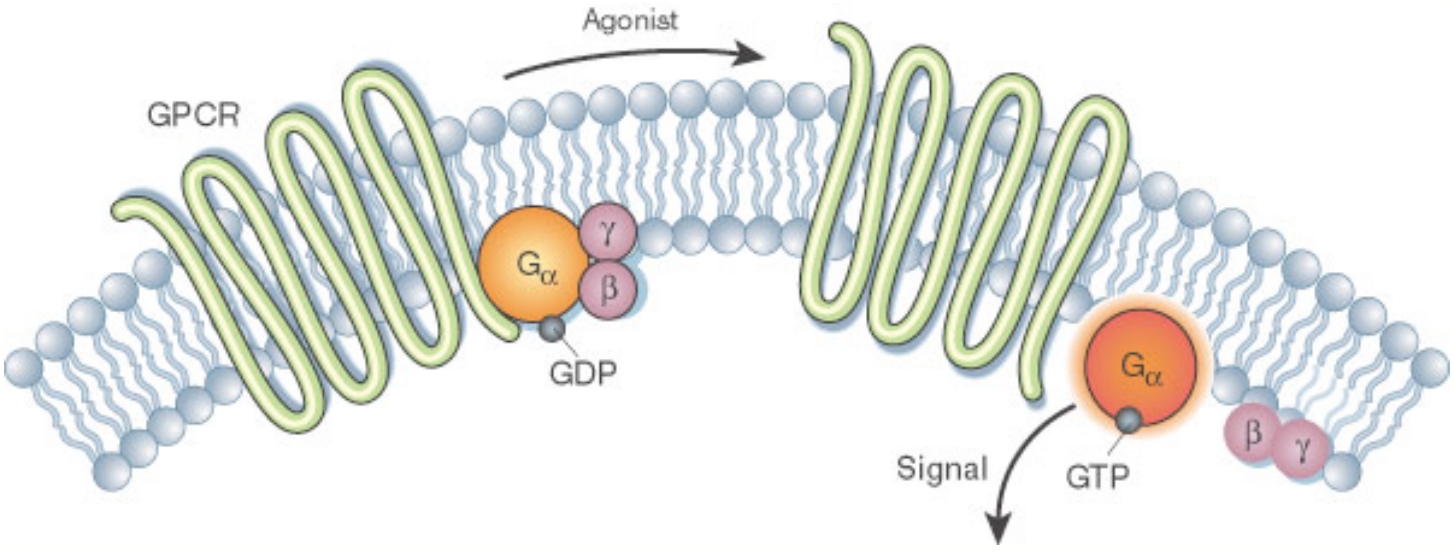


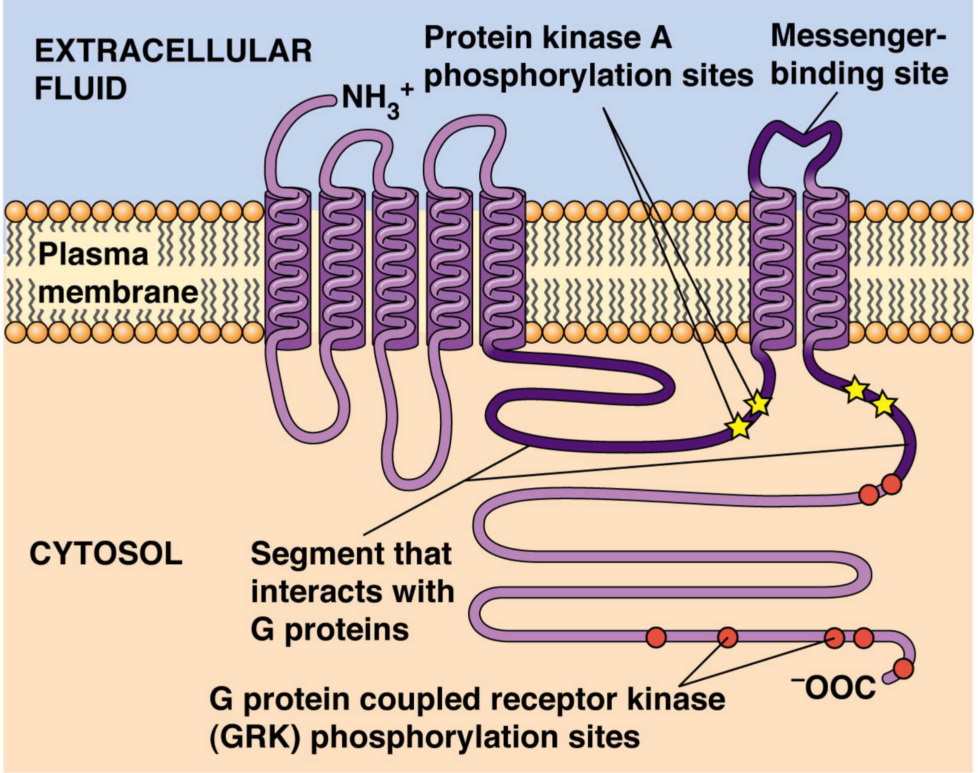
Image credit: creative-diagnostics.com

Regulation of G protein-linked receptors

- G protein-linked receptors can be regulated in several ways; It is important for a cell to be able to stop them from activating G proteins.

  - Phosphorylation of amino acids in the cytosolic domain, carried out by **G protein-linked receptor kinases** (**GRKs**), which act on activated receptors

  - Desensitization, or adaptation to a persistent stimulus
    - E.g. by β-arrestin
    - Arrestin binding to the receptor blocks further G protein-mediated signaling and targets receptors for internalization


EXTRACELLULAR FLUID
NH_3^+
Protein kinase A phosphorylation sites
Messenger-binding site
Plasma membrane
CYTOSOL
Segment that interacts with G proteins
G protein coupled receptor kinase (GRK) phosphorylation sites
^-OOC

G protein Inactivation

- G proteins remain active as long as the G_{α} subunit is bound to GTP and separate from the $G_{\beta\gamma}$ subunit
- Once the G_{α} subunit has hydrolyzed GTP to GDP, it re-associates with $G_{\beta\gamma}$

- Some G_{α} proteins are not very efficient at GTP hydrolysis (i.e. turning off) so they sometimes have help…
- G protein activity (for GTP hydrolysis) is greatly enhanced by regulators of G protein-signaling (RGS) proteins

- The most important G protein function is release or formation of second messengers

https://www.youtube.com/watch?v=Glu_T6DQuLU

Cyclic AMP Is a Second Messenger Whose Production Is Regulated by Some G Proteins

- **Cyclic AMP** (**cAMP**) is formed from cytosolic ATP by **adenylyl cyclase**, an enzyme that is anchored in the plasma membrane
- The enzyme is inactive until bound to activated $Gs_{\alpha;}$ (by receptor-ligand stimulated acquisition of GTP and release from $Gs_{\beta\gamma}$)

G proteins are active for only a short time

- Because G proteins remain active for a very short time, they can respond quickly to changing conditions
  - Once a G protein becomes inactive, adenylyl cyclase stops making new cAMP
  - The cAMP that remains is degraded
- cAMP is important in many cellular events
- Its main target is protein kinase A (PKA)
- PKA phosphorylates a variety of proteins, using ATP as the source of phosphate
- **When cAMP levels are reduced, PKA activation is also reduced, therefore phosphorylation of cytosolic GPCR sites slows**

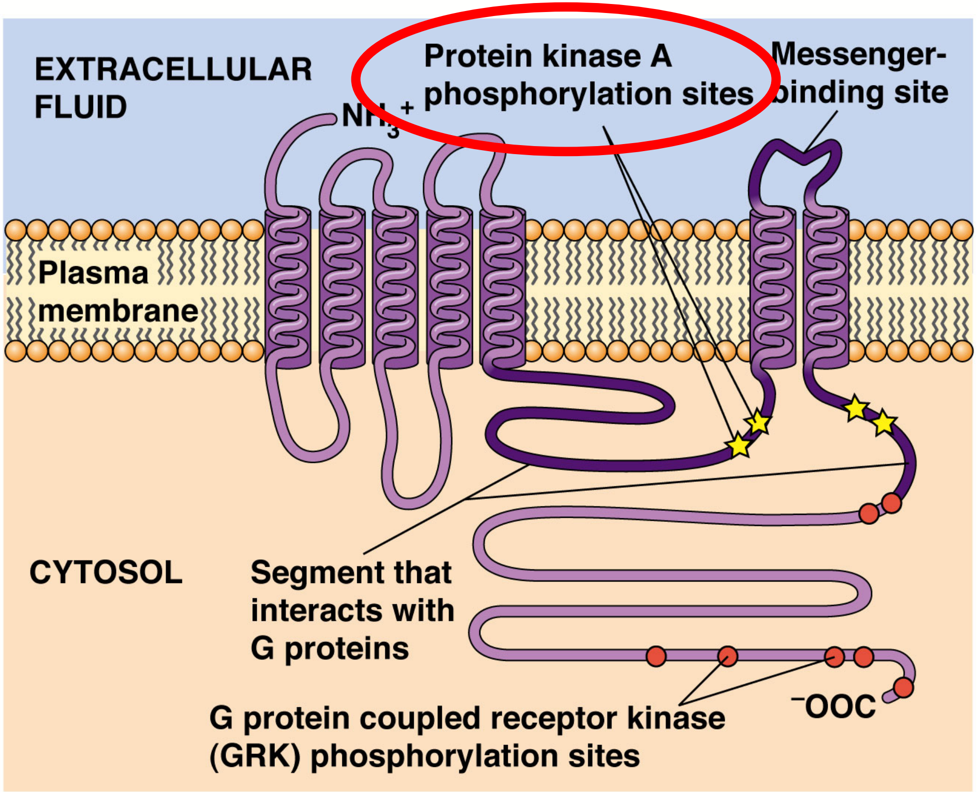

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Table 14-1 **Examples of Cell Functions Regulated by cAMP**

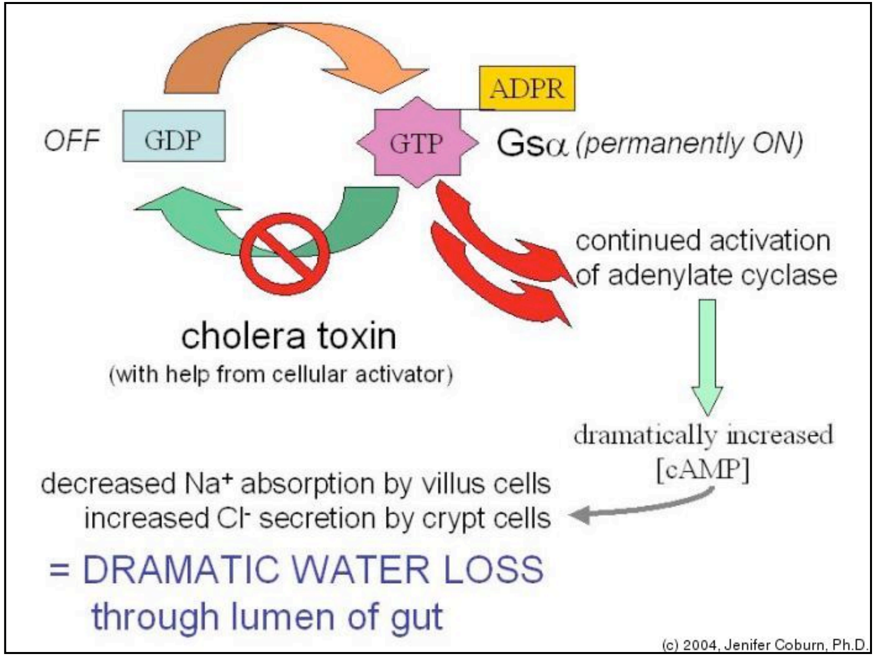
Regulated Function	Target Tissues	Hormone
Glycogen degradation	Muscle, liver	Epinephrine
Fatty acid production	Adipose	Epinephrine
Heart rate, blood pressure	Cardiovascular	Epinephrine
Water reabsorption	Kidney	Antidiuretic hormone
Bone resorption	Bone	Parathyroid hormone

Disruption of G Protein Signaling Causes Several Human Diseases

Why is it important that G proteins are only active for a short time?

Examples seen in human disease:

1. **Cholera** *(Vibrio cholerae)*
2. **Whooping cough** *(Bordetella pertussis)*



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Many G Proteins Use Inositol Trisphosphate and Diacylglycerol as Second Messengers

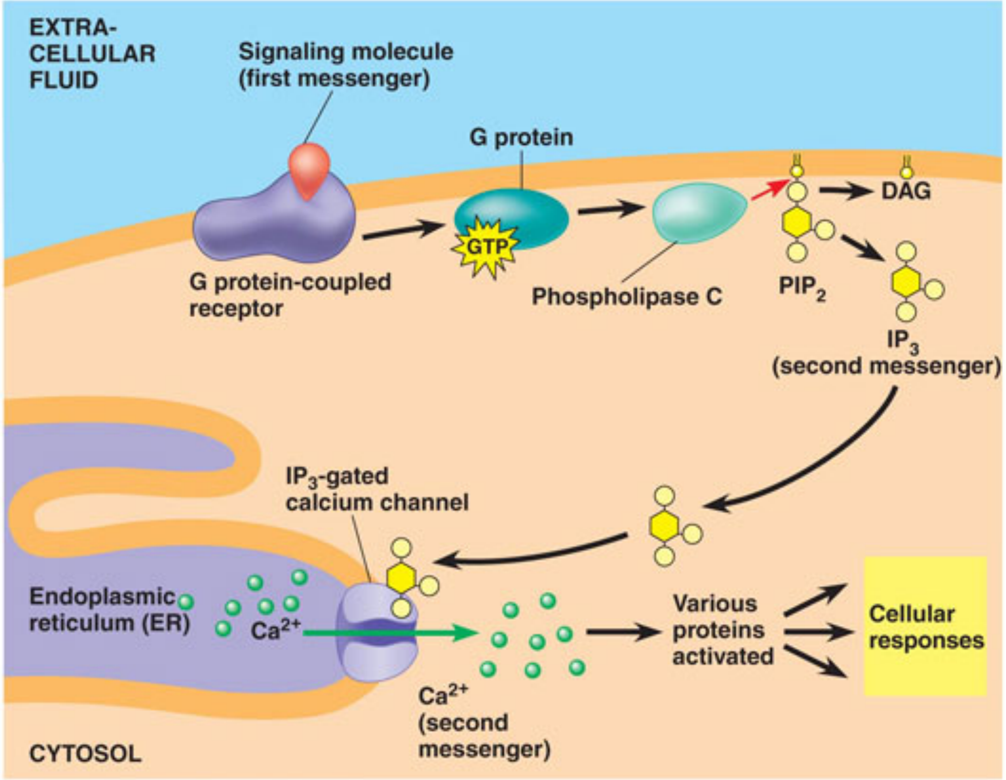


Image credit: U. Miami Dept. of Biology

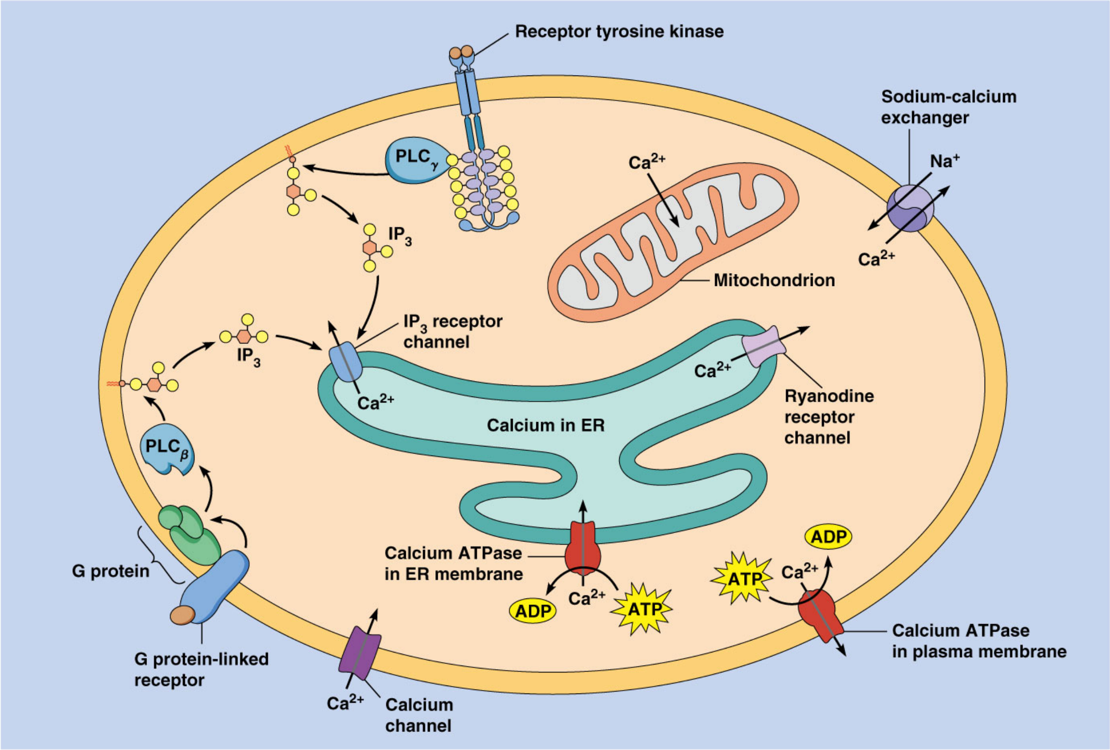
Table 14-2 **Examples of Cell Functions Regulated by Inositol Trisphosphate and Diacylglycerol**

Regulated Function	Target Tissues	Messenger
Platelet activation	Blood platelets	Thrombin
Muscle contraction	Smooth muscle	Acetylcholine
Insulin secretion	Pancreas, endocrine	Acetylcholine
Amylase secretion	Pancreas, exocrine	Acetylcholine
Glycogen degradation	Liver	Antidiuretic hormone
Antibody production	B lymphocytes	Foreign antigens

Calcium in signaling

- Ca^{2+} plays an essential role in regulating a variety of cellular functions
- Calcium concentrations are maintained at low levels through **calcium ATPases** in the plasma membrane and ER; these transport calcium ions out of the cytosol
- Calcium concentrations can be released by opening calcium channels in the plasma membrane, as in neuronal signaling
- Calcium can also be released from storage in the ER through the IP_3 receptor channel
- Calcium-sensitive fluorescent dyes (**calcium indicators**) can be used to demonstrate the importance of calcium release in signaling

https://www.youtube.com/watch?v=lsYBeFqEwzk


Receptor tyrosine kinase
Sodium-calcium exchanger
Na+
Ca2+
PLCγ
Ca2+
IP3
Mitochondrion
IP3 receptor channel
IP3
Ca2+
Ryanodine receptor channel
Ca2+
Calcium in ER
PLCβ
ADP
Calcium ATPase in ER membrane
ATP
Ca2+
G protein
ADP
Ca2+
ATP
Calcium ATPase in plasma membrane
G protein-linked receptor
Ca2+
Calcium channel

Summary

- Receptor categories
- GPCRs – G-protein coupled receptors
  - Structure
  - Regulation
  - Inactivation
- Second Messengers
  - cAMP
  - Calcium ions

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