

# 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

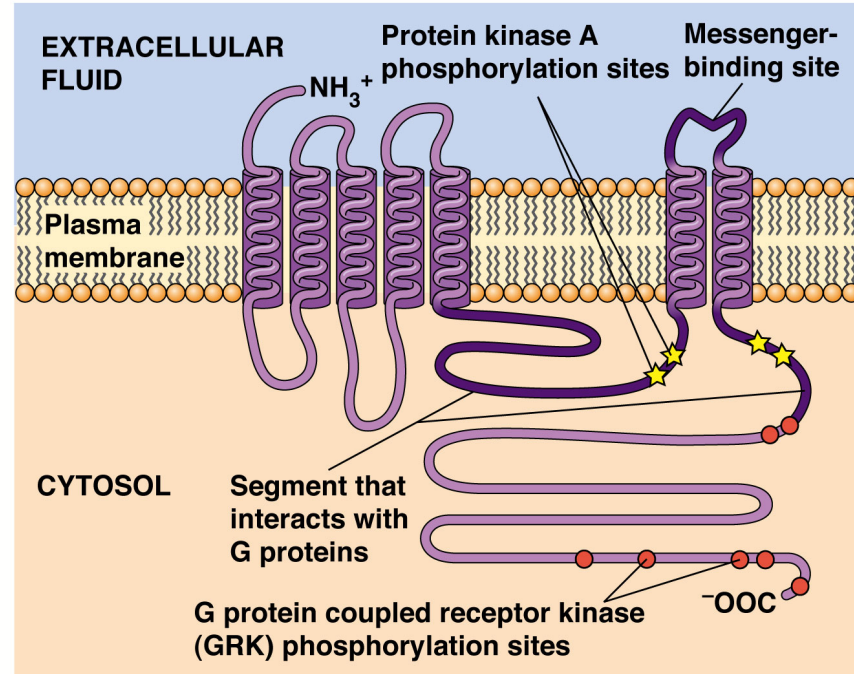
- Receptors can be classified into several basic categories
  - Ligand-gated channels (e.g. ion channels that allow neurotransmitters to pass through)
  - Plasma membrane receptors of two types
    - **Those linked to G proteins**
    - Those linked to protein kinases

# 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  $\alpha$  helices connected by alternating cytosolic or extracellular loops
- The extracellular portion of each receptor has a unique messenger-binding site



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# 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  $G_{\alpha}$ ,  $G_{\beta}$ , and  $G_{\gamma}$  subunits

# G protein Structure & Function

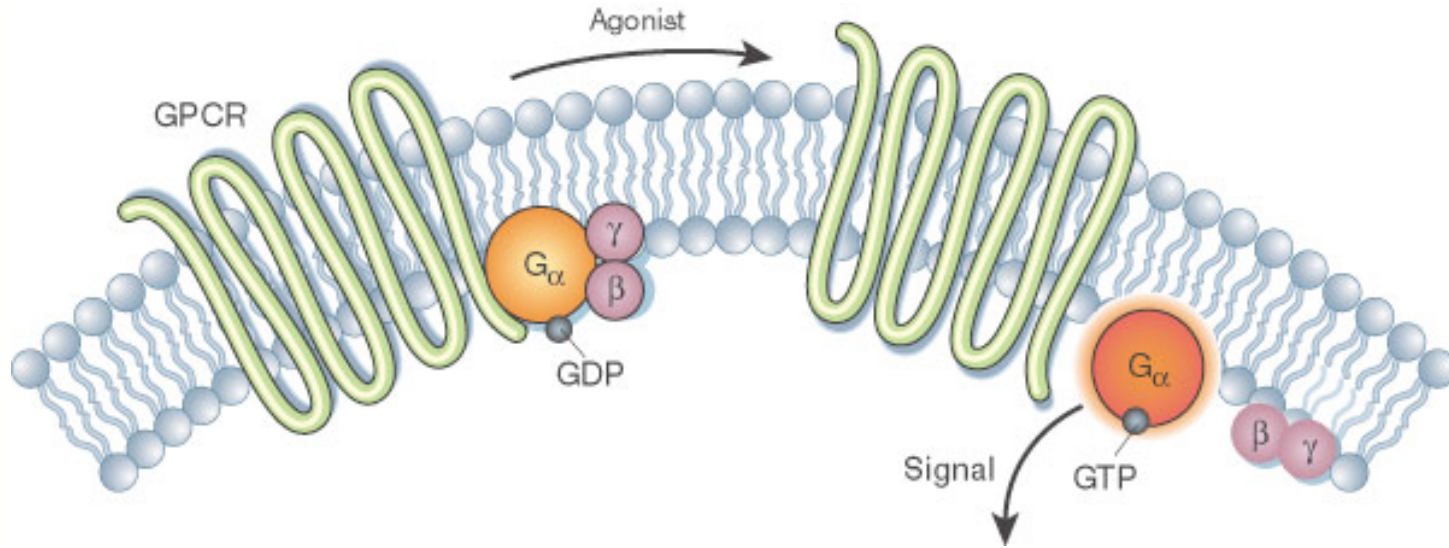
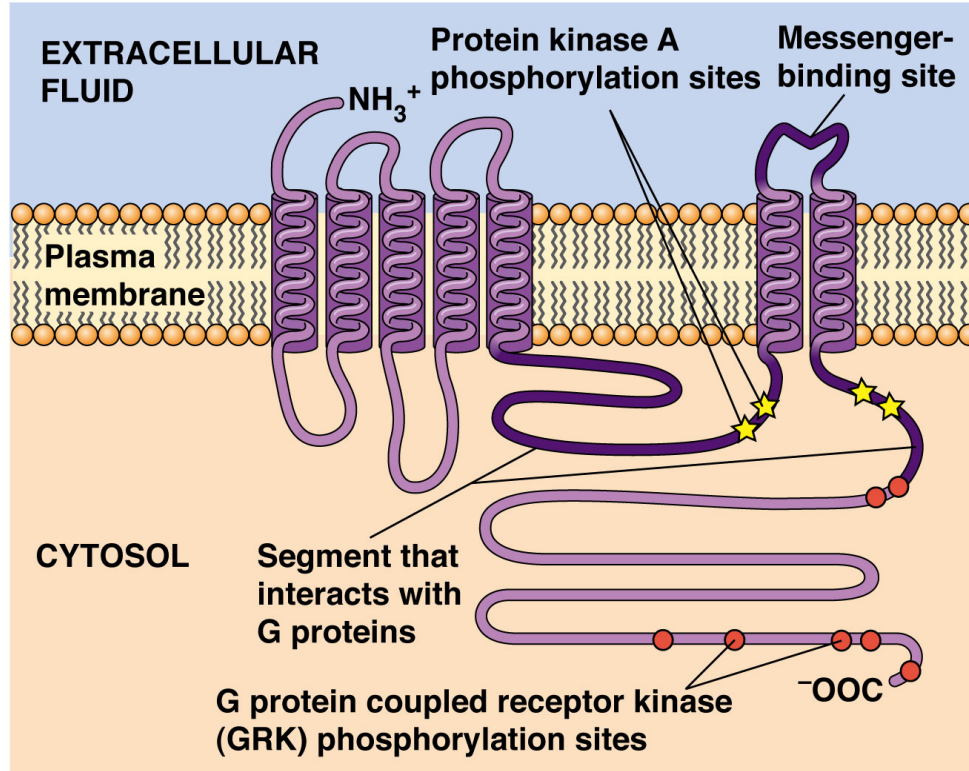


Image credit: creative-diagnostics.com

# 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  $\beta$ -arrestin
  - Arrestin binding to the receptor blocks further G protein-mediated signaling and targets receptors for internalization





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# G protein Inactivation

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

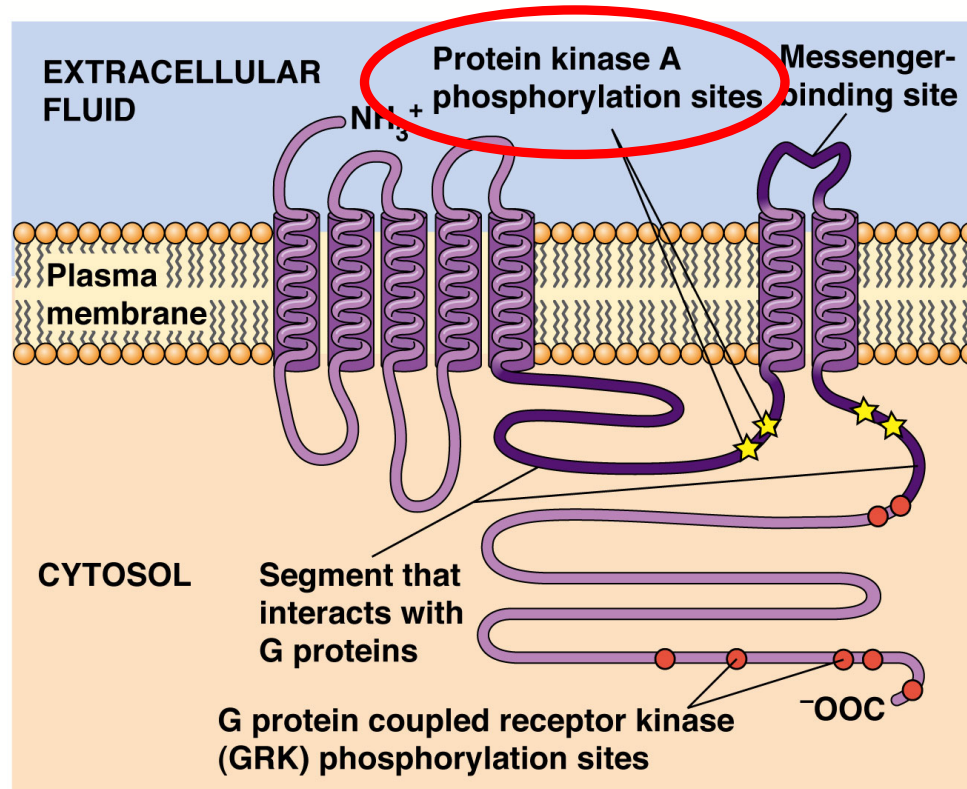
[https://www.youtube.com/watch?v=Glu\\_T6DQuLU](https://www.youtube.com/watch?v=Glu_T6DQuLU)

# 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  $G_{s\alpha}$ ; (by receptor-ligand stimulated acquisition of GTP and release from  $G_{s\beta\gamma}$ )

# 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**

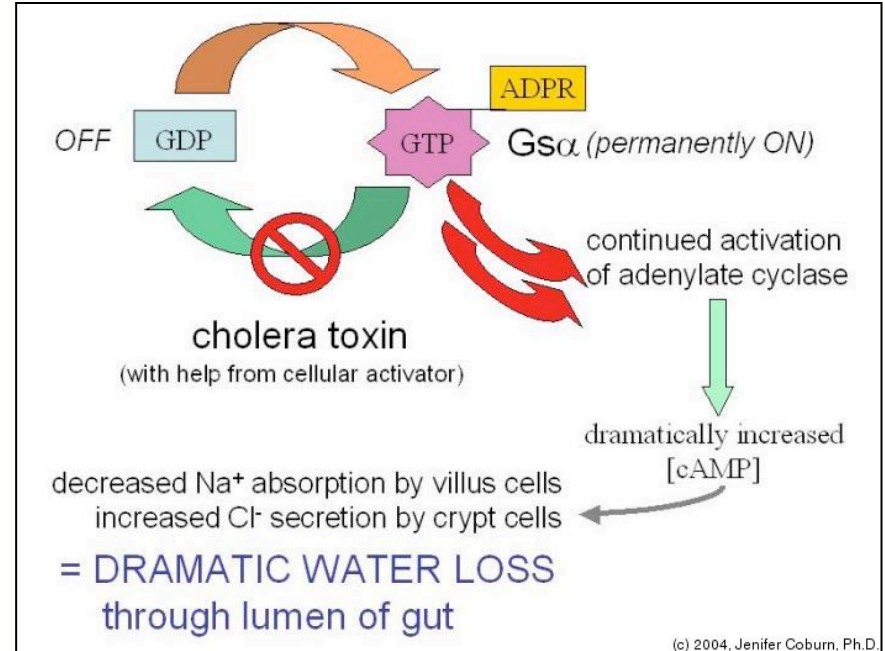
<b>Regulated Function</b>	<b>Target Tissues</b>	<b>Hormone</b>
Glycogen degradation	Muscle, liver	Epinephrine
Fatty acid production	Adipose	Epinephrine
Heart rate, blood pressure	Cardiovascular	Epinephrine
Water reabsorption	Kidney	Antidiuretic hormone
Bone resorption	Bone	Parathyroid hormone

# Disruption of G Protein Signaling Causes Several Human Diseases

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

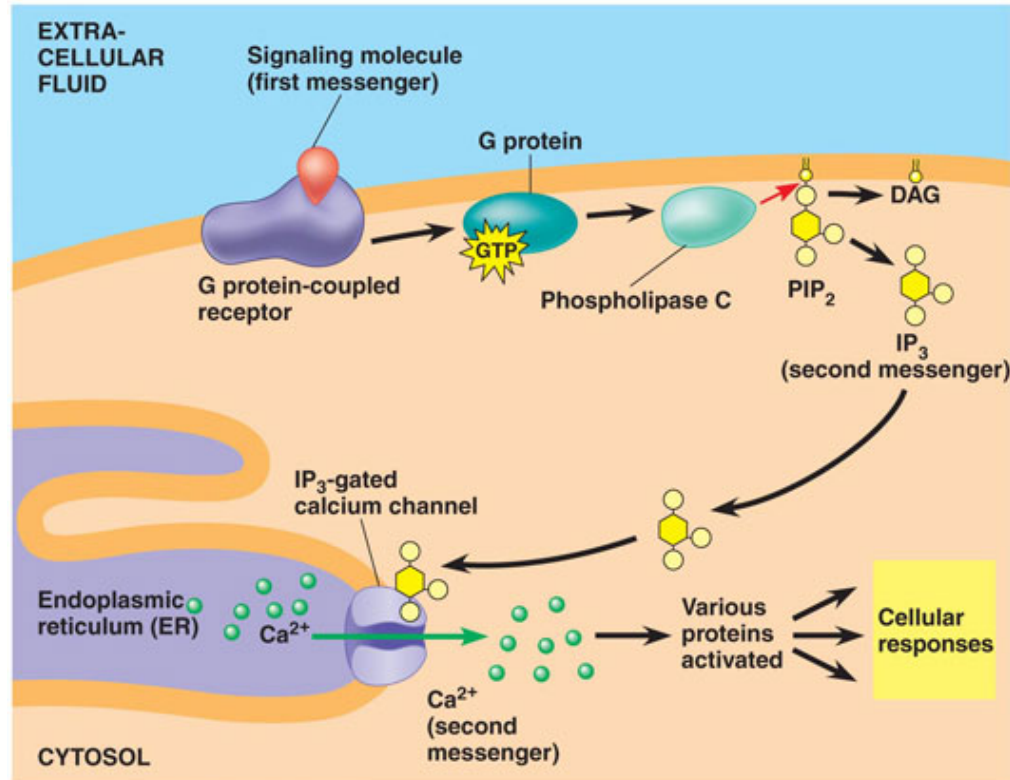
Examples seen in human disease:

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





# Many G Proteins Use Inositol Trisphosphate and Diacylglycerol as Second Messengers



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Image credit: U. Miami Dept. of Biology

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

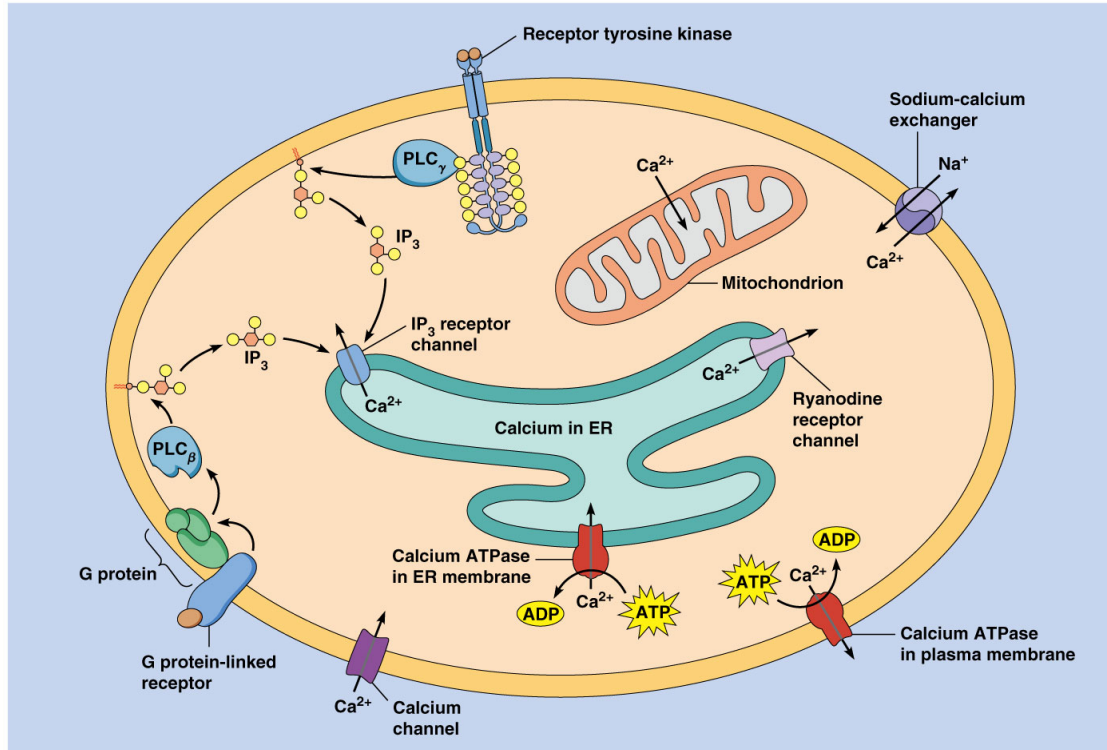
<b>Regulated Function</b>	<b>Target Tissues</b>	<b>Messenger</b>
Platelet activation	Blood platelets	Thrombin
Muscle contraction	Smooth muscle	Acetylcholine
Insulin secretion	Pancreas, endocrine	Acetylcholine
Amylase secretion	Pancreas, exocrine	Acetylcholine
Glycogen degradation	Liver	Antidiuretic hormone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

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# Calcium in signaling

- $\text{Ca}^{2+}$  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  $\text{IP}_3$  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=IsYBeFqEwzk>



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# Summary

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



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