**G-protein linked receptors**

- A very large family of receptors coupled to trimeric G proteins
- Activate or inhibit adenylyl cyclase, or activate phospholipase C
- All have seven membrane spanning region
- Ligands include:
  - Hormones, neurotransmitters, light activated receptors (rhodopsins), thousands of odorant receptors
Second messengers are small, nonprotein, water-soluble molecules or ions.

The extracellular signal molecule that binds to the membrane is a pathway’s “first messenger.”

Second messengers can readily spread throughout cells by diffusion.

Second messengers participate in pathways initiated by G-protein-linked receptors and receptor tyrosine kinases.
3',5'-Cyclic GMP (cGMP)
Activates protein kinase G (PKG) and opens cation channels in rod cells

3',5'-Cyclic AMP (cAMP)
Activates protein kinase A (PKA)

Inositol 1,4,5-trisphosphate (IP₃)
Opens Ca²⁺ channels in the endoplasmic reticulum

1,2-Diacylglycerol (DAG)
Activates protein kinase C (PKC)

N-ethylmaleimide-sensitive factor (NSF)
Fat acyl groups
Glycerol
• Multistep pathways have two important benefits:
  - Amplifying the signal (and thus the response)
  - Contributing to the specificity of the response
  - At each step, the number of activated products is much greater than in the preceding step
• Signal transducing $G$ protein has 3 subunits
  - $G\alpha$, $G\beta$ and $G\gamma$
• $G\alpha$ is the GTPase switch protein
• modulates the activity of an effector protein
• Effector proteins are either membrane bound ion channels or enzymes generating second messengers

• adenylate cyclase  $(G_s, G_i)$
  - produces cyclic AMP (cAMP)
• guanyl cyclase
  - produces cyclic GMP (cGMP)
• phospholipase C $(G_p)$
  - produces inositol trisphosphate (IP$_3$) and diacyl glycerol (DAG)
• ion channels
Function of $G_s$-protein in activation of adenylate cyclase

1. **Unbound State**
   - Receptor (R) is not engaged with the $G_s$-protein complex.
   - $G_s$-protein complex is in the GDP-bound state.
   - Adenylate cyclase (AC) is not activated.

2. **Stimulation**
   - Receptor (R) binds to the $G_s$-protein complex.
   - This leads to the release of GDP and binding of GTP in the $G_s$-protein complex.
   - GTP stimulates AC to increase the production of cAMP.

3. **Inactivation**
   - GTP-bound $G_s$-protein complex can rebind GDP, reversing the activation process.
   - This cycle allows for the regulation of AC activity in response to receptor stimulation.
Reception

Binding of epinephrine to G-protein-linked receptor (1 molecule)

Transduction

Inactive G protein

Active G protein ($10^2$ molecules)

Inactive adenylyl cyclase

Active adenylyl cyclase ($10^2$)

ATP

Cyclic AMP ($10^8$)

Inactive protein kinase A

Active protein kinase A ($10^4$)

Inactive phosphorylase kinase

Active phosphorylase kinase ($10^9$)

Inactive glycogen phosphorylase

Active glycogen phosphorylase ($10^6$)

Response

Glycogen

Glucose-1-phosphate ($10^8$ molecules)
Synthesis and degradation of cAMP

1. ATP + adenylyl cyclase → cAMP
2. cAMP + cyclic AMP phosphodiesterase → 5’-AMP
| Adipose tissue | epinephrine, ACTH, glucagon | lipolysis, decrease of lipogenesis |
| Liver | epinephrine, norepinephrine, glucagon | glycogenolysis, inhibition of glycogen synthesis, gluconeogenesis |
| Adrenal cortex | ACTH | synthesis of glucocorticoids, secretion of thyroxin |
| Thyroid gland | TSH | synthesis of estrogens, progesterone, increase of contraction rate |
| Ovarian follicle | FSH, LH | increase of calcium resorption from bone, resorption of water |
| Cardiac muscle | epinephrine | fluid secretion, inhibition of platelet aggregation and secretion |
| Bone cells | parathormone | |
| Kidney | vasopressin | |
| Intestine | epinephrine | |
| Blood platelets | prostaglandin I | |
Phosphatidylinositols and synthesis of 2nd messengers DAG and IP₃
Protein Kinase C Activation of Gene Transcription

Two Independent Pathways
**IP$_3$ + DAG and their roles in regulation**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Signal</th>
<th>Receptor</th>
<th>Effects</th>
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<tr>
<td>Smooth muscle</td>
<td>Acetylcholine</td>
<td>$M_1$</td>
<td>Contraction</td>
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<td>Epinephrine</td>
<td>$\alpha_1$</td>
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<tr>
<td>Smooth muscles</td>
<td>Vasopresin</td>
<td>$V_2$</td>
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<td>Activation of PLA$_2$</td>
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**INTRACELLULAR Ca\(^{2+}\) AS A “SECOND” MESSENGER**

Cells maintain low intracellular [Ca\(^{2+}\)] in order for \(\uparrow\) Ca\(^{2+}\) to result in effective intracellular signaling.

Cellular mechanisms that maintain very low intracellular Ca\(^{2+}\) concentrations

**A:** Ca\(^{2+}\) is actively pumped out of the cytosol.

**B:** Ca\(^{2+}\) is pumped into the ER and mitochondria.
Calcium as second messenger

- all eukaryotic cells maintain free intracellular calcium at nanomolar concentrations
- the sole function of calcium is to transmit information. This is carried out by Ca increase to uM concentrations
- this Ca increase results in specific binding to several different proteins in the cell
- these proteins have specific “pockets” for Ca with similar amino acid domains
• plasma membranes have low permeability to Ca so that concentration gradients of 5 orders of magnitude are achieved (50 nM inside vs 1.3 mM outside)
• the total Ca in the cell is much higher but it is the free Ca which oscillates and is regulated
• the major pathways for cellular Ca increase are:
  - voltage sensitive Ca channels
  - receptor dependent Ca channels
  - Na-Ca exchange
  - Ca ATPase of sarco(endo)plasmic reticulum
  - Ca ATPase of plasma membranes
  - Ca releasing channels of the sarcoplasmic or endoplasmic reticulum
Release from intracellular stores subsequent to PLC-catalyzed hydrolysis of PIP$_2$

Increased intracellular [Ca$^{2+}$] - “3$^{rd}$ messenger”: Immediate vs. Sustained responses
- Ca$^{2+}$ binds to its ubiquitous intracellular receptor, calmodulin, thereby
  a) activatingCa$^{2+}$/calmodulin-dependent kinases (CaM kinases), Ser/Thr kinases
  b) phopshorylation of proteins, some of which when phosphorylated can ↑ or ↓ gene transcription.

Must be distinguished from cAMP-induced effects where cAMP activates a variety of Ca$^{2+}$ channels → ↑ Ca$^{2+}$ influx from extracellular environment
  e.g. β-adrenergic receptor occupancy
    in myocardium → ↑ Ca$^{2+}$ influx → ↑ rate and force of heart beat).