Signal Transduction

Lect 3

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• Membrane receptors

Membrane Glycoprotein

• Intracellular receptors

Cytosol or nuclei

DNA binding protein
Receptors superfamilies:
- Ionotropic receptors (ligand-gated channels)
- Metabotropic receptors (G protein-coupled receptors)
- Tyrosine Kinase

Almost all neurotransmitters discovered so far have more than one kind of receptor -- called **receptor subtypes**.
Ionotropic Receptors

Work very fast; important role in fast neurotransmission

1. Each is made of several subunits (together form the complete receptor)
2. At center of receptors is channel or pore to allow flow of ions
3. At rest - receptor channels are closed
4. When neurotransmitter binds -- channel immediately opens
5. When ligand leaves binding site -- channel quickly closes
Metabotropic Receptors...

Work by activating other proteins called **G proteins**

1. Each is made of several transmembrane regions

2. Stimulate or inhibit the opening of ion channels in the cell membrane

3. Work more slowly than ionotrophic receptors but lasts longer
1. Stimulate or inhibit certain effector enzymes
2. Most effector enzymes controlled by G proteins are involved in synthesis of second messengers.

*First messenger: ligand.
*Second messenger: effector enzyme
3. Three major classes of surface receptors for signaling:

(A) ION-CHANNEL-LINKED RECEPTORS

(B) G-PROTEIN-LINKED RECEPTORS

(C) ENZYME-LINKED RECEPTORS

Receptors determine response

No receptor - no response
3. Three major classes of surface receptors for signaling, cont.:
   A. Ion Channels:
   B. G protein-coupled receptors (GPRs): largest family of cell surfacereceptors; present in all eukaryotes; ex: adrenergic receptors, opioid receptors.

   1. Overview:
      a. 7 trans-membrane spanning domains
      b. Act as receptors for many different ligands including NT, H
      c. Large amount of receptor diversity, but common mechanism of action
      d. Transmit signals to intracellular targets via G proteins
      e. Targets are plasma membrane bound enzymes or ion channels

   2. Mechanism of Activation of GPRs:
      a. Binding of ligand to extracellular domain of GPRs induces conformational change that allows cytosolic domain of the receptor to bind to inactive G protein at inner face of PM.
      b. This interaction activates the G protein, which dissociates from the receptor.
      c. Activated G protein α subunit can now bind GTP instead of GDP, causing dissociation into activated α vs. βγ subunits. Each of these can go on to activate target proteins.

   C. Enzyme-linked receptors:
G Protein Signal Cascade

The **signal** is usually passed from a **7-helix receptor** to an intracellular **G-protein**.

- Seven-helix receptors are thus called **GPCR**, or **G-Protein-Coupled Receptors**.
- Approx. 800 different GPCRs are encoded in the human genome.
1. Binding of hormone induces a conformational change in receptor

2. Activated receptor binds to $G_\alpha$ subunit

3. Activated receptor causes conformational change in $G_\alpha$, triggering dissociation of GDP

4. Binding of GTP to $G_\alpha$, triggering dissociation of $G_\alpha$ both from the receptor and from $G_{\beta\gamma}$

5. Hormone dissociates from receptor; $G_\alpha$ binds to effector, activating it

6. Hydrolysis of GTP to GDP causes $G_\alpha$ to dissociate from effector and reassociate with $G_{\beta\gamma}$
G Protein Signal Cascade

- **G-proteins** are heterotrimeric, with 3 subunits $\alpha$, $\beta$, $\gamma$.

- A G-protein that activates cyclic-AMP formation within a cell is called a **stimulatory G-protein**, designated $G_s$ with alpha subunit $G_{s\alpha}$.

- $G_s$ is activated, e.g., by receptors for the hormones epinephrine and glucagon.

The **$\beta$-adrenergic receptor** is the **GPCR** for epinephrine.
<table>
<thead>
<tr>
<th>( \alpha ) CLASS</th>
<th>ASSOCIATED EFFECOR</th>
<th>2ND MESSENGER</th>
<th>RECEPTOR EXAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )s</td>
<td>Adenylyl cyclase</td>
<td>cAMP (increased)</td>
<td>( \beta )-Adrenergic (epinephrine) receptor; receptors for glucagon, serotonin, vasopressin</td>
</tr>
<tr>
<td>( \alpha )i</td>
<td>Adenylyl cyclase</td>
<td>cAMP (decreased)</td>
<td>( \alpha_2 )-Adrenergic receptor</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>K+ channel (( \beta \gamma ) activates effector)</td>
<td>Change in membrane potential</td>
<td>Muscarinic acetylcholine receptor</td>
</tr>
<tr>
<td>( \alpha )olf</td>
<td>Adenylyl cyclase</td>
<td>cAMP (increased)</td>
<td>Odorant receptors in nose</td>
</tr>
<tr>
<td>( \alpha )q</td>
<td>Phospholipase C</td>
<td>IP( _3 ), DAG (increased)</td>
<td>( \alpha_1 )-Adrenergic receptor</td>
</tr>
<tr>
<td>( \alpha )o</td>
<td>Phospholipase C</td>
<td>IP( _3 ), DAG (increased)</td>
<td>Acetylcholine receptor in endothelial cells</td>
</tr>
<tr>
<td>( \alpha )t</td>
<td>cGMP phosphodiesterase</td>
<td>cGMP (decreased)</td>
<td>Rhodopsin (light receptor) in rod cells</td>
</tr>
</tbody>
</table>

*A given \( \alpha \) subclass may be associated with more than one effector protein. To date, only one major \( \alpha_s \) has been identified, but multiple \( \alpha_i \) and \( \alpha \) proteins have been described. Effector proteins commonly are regulated by \( \alpha \) but in some cases by \( \beta \gamma \) or the combined action of \( \alpha \) and \( \beta \gamma \).

IP\( _3 \) = inositol 1,4,5-trisphosphate; DAG = 1,2-diacylglycerol.


Table 15-1
Molecular Cell Biology, Sixth Edition
Summary of Hormones signaling pathways

<table>
<thead>
<tr>
<th></th>
<th>IP&lt;sub&gt;3&lt;/sub&gt;</th>
<th>cAMP</th>
<th>cGMP</th>
<th>Tyrosine kinase - intrinsic</th>
<th>Tyrosine kinase - receptor associated</th>
<th>Steroid</th>
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<tbody>
<tr>
<td>GnRH</td>
<td>FSH</td>
<td>ANP</td>
<td></td>
<td>Insulin</td>
<td>Prolactin</td>
<td>Glucocorticoid</td>
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<tr>
<td>Gastrin</td>
<td>LH</td>
<td>NO (EDRF)</td>
<td></td>
<td>IGF-1</td>
<td>Cytokines (IL-2,6,8)</td>
<td>Estrogen</td>
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<tr>
<td>Oxytocin</td>
<td>ACTH</td>
<td></td>
<td></td>
<td>FGF</td>
<td>GH</td>
<td>Progesterone</td>
</tr>
<tr>
<td>TRH</td>
<td>TSH</td>
<td></td>
<td></td>
<td>PDGF</td>
<td></td>
<td>Testosterone</td>
</tr>
<tr>
<td>ADH (V₃)</td>
<td>CRH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aldosterone</td>
</tr>
<tr>
<td>Histamine (H&lt;sub&gt;₁&lt;/sub&gt;)</td>
<td>hCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vitamin D</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>PTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>T₃/T₄</td>
</tr>
<tr>
<td>Calcitonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucagon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cortisol</td>
</tr>
<tr>
<td>GHRH (can act via IP&lt;sub&gt;3&lt;/sub&gt; as well)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
• The $\alpha$ subunit of a G-protein ($G_\alpha$) binds GTP, & can hydrolyze it to GDP + P$_i$.

$\alpha$ & $\gamma$ subunits have covalently attached lipid anchors that bind a G-protein to the plasma membrane cytosolic surface.

• Adenylate Cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.
Adenylate Cyclase (Adenylyl Cyclase) catalyzes:

$$\text{ATP} \rightarrow \text{cAMP} + \text{PP}_i$$

Binding of certain **hormones** (e.g., epinephrine) to the outer surface of a cell activates Adenylate Cyclase to form cAMP within the cell.

Cyclic AMP is thus considered to be a **second messenger**.
G Protein Signal Cascade

The **sequence of events** by which a hormone activates cAMP signaling:

1. Initially $G_\alpha$ has bound GDP, and $\alpha$, $\beta$, & $\gamma$ subunits are complexed together. $G_{\beta,\gamma}$, the complex of $\beta$ & $\gamma$ subunits, inhibits $G_\alpha$. 

![Diagram of G Protein Signal Cascade](image-url)
2. **Hormone binding**, usually to an extracellular domain of a 7-helix receptor (GPCR), causes a *conformational change* in the receptor that is transmitted to a *G protein* on the cytosolic side of the membrane. The nucleotide-binding site on \( G_\alpha \) becomes more accessible to the cytosol, where \([\text{GTP}] > [\text{GDP}]\). \( G_\alpha \) releases GDP & binds GTP (GDP-GTP exchange).
3. Substitution of GTP for GDP causes another conformational change in Gα.

Gα-GTP dissociates from the inhibitory βγ complex & can now bind to and activate Adenylate Cyclase.
4. **Adenylate Cyclase**, activated by the stimulatory G\(_{\alpha}\)-GTP, catalyzes synthesis of cAMP.

5. **Protein Kinase A** (cAMP Dependent Protein Kinase) catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.
Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:

- Some protein kinases are activated by Ca\(^{++}\)-calmodulin.
- **Protein Kinase A** is activated by cyclic-AMP (cAMP).

**Protein Kinase A** (cAMP-Dependent Protein Kinase) transfers Pi from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

Protein Kinase A in the resting state is a complex of:
- 2 catalytic subunits (C)
- 2 regulatory subunits (R).

R\(_2\)C\(_2\) : When each (R) binds 2 cAMP, a conformational change causes (R) to release (C).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.

**PKIs**, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (C).
Turn off of the signal:

1. \( G_\alpha \) hydrolyzes GTP to GDP + P\(_i\). (GTPase).
   
The presence of GDP on \( G_\alpha \) causes it to rebind to the inhibitory \( \beta\gamma \) complex.
   
   Adenylate Cyclase is no longer activated.

2. **Phosphodiesterases** catalyze hydrolysis of cAMP \( \rightarrow \) AMP.
Phosphodiesterase enzymes catalyze:

\[ \text{cAMP} + \text{H}_2\text{O} \rightarrow \text{AMP} \]

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus cAMP stimulates its own degradation, leading to rapid turnoff of a cAMP signal.
3. **Receptor desensitization** varies with the hormone.
- In some cases the **activated receptor** is **phosphorylated** via a G-protein Receptor Kinase.
- The phosphorylated receptor then may bind to a protein **β-arrestin**.
- **β-Arrestin** promotes removal of the receptor from the membrane by clathrin-mediated endocytosis.
- **β-Arrestin** may also bind a cytosolic **Phosphodiesterase**, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.
Different isoforms of $G_\alpha$ have different signal roles. E.g.:

- The **stimulatory** $G_{s\alpha}$, when it binds GTP, **activates** Adenylate cyclase.

- An **inhibitory** $G_{i\alpha}$, when it binds GTP, **inhibits** Adenylate cyclase.

The complex of $G_{\beta,\gamma}$ that is released when $G_\alpha$ binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.

E.g., $G_{\beta,\gamma}$ **inhibits** one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme.
Figure 15-21