Receptors and Protein-Protein Interaction
-----signal transduction proteins

♣. Cellular activities are regulated by cell surface receptors and other intracellular signaling proteins.

ECM: ExtraCellular Matrix

♣. Different cells have different ECM

♣. Protein-protein interaction plays a very important role in signal transduction.

♣. Many diseases are directly caused by erratic signaling.
Intracellular signaling is also very important of regulation of cellular functions.
1. Primary messengers.

A. Hormones

- Epinephrine
- Cortisol

B. Proteins: Epidermal Growth Factor (EGF)

C. Other stimuli:

- Many other growth factors, Interferon, insulin, hGH, etc.

- Many others

such as light
2. Receptor 1:
Epidermal growth factor (EGFR), single transmembrane protein

Tyrosine kinases form a receptor superfamily
2. Receptor 2:
human growth hormone receptor (hGHR), single transmembrane protein

[Diagram showing hGHR binding hGH, leading to activation of JAK2 and STAT pathway]
2. Receptor 3: insulin receptor (INSR), dimeric transmembrane protein
2. Receptor 4: GABA receptor, dimeric protein ($\alpha_2\beta_2$) with 4 membrane-spanning helices per subunit.

Chemical synapse: signaling by neurotransmitters
2. Receptor 5:  
Adrenergic receptor, single protein with 7 membrane-spanning helices

Typical of G-protein coupled receptors, another superfamily of signaling proteins.
G-proteins: guanyl nucleoside binding (GNP) proteins

GPCR: G-protein coupled receptors

Table 38-2
Physiological processes mediated by G proteins

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Receptor</th>
<th>G protein</th>
<th>Effector</th>
<th>Physiological response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>β-Adrenergic receptor</td>
<td>$G_s$</td>
<td>Adenylate cyclase</td>
<td>Glycogen breakdown</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Serotonin receptor</td>
<td>$G_s$</td>
<td>Adenylate cyclase</td>
<td>Behavioral sensitization and learning in <em>Aplysia</em></td>
</tr>
<tr>
<td>Light</td>
<td>Rhodopsin</td>
<td>Transducin</td>
<td>cGMP phosphodiesterase</td>
<td>Visual excitation</td>
</tr>
<tr>
<td>IgE-antigen complexes</td>
<td>Mast cell IgE receptor</td>
<td>$G_{PLC}$</td>
<td>Phospholipase C</td>
<td>Secretion</td>
</tr>
<tr>
<td>f-Met peptide</td>
<td>Chemotactic receptor</td>
<td>$G_{PLC}$</td>
<td>Phospholipase C</td>
<td>Chemotaxis</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Muscarinic receptor</td>
<td>$G_K$</td>
<td>Potassium channel</td>
<td>Slowing of pacemaker activity</td>
</tr>
</tbody>
</table>

Note: $G_{PLC}$ and $G_K$ refer to as yet unidentified G proteins in these cascades.
2. Receptor 6:

- Light receptor
- 7 membrane-spanning helices

Mediated by bound retinal (Vitamin A)

![Diagram showing light absorption process](image)

- Rhodopsin (500 nm)
- Bathorhodopsin (543 nm)
- Lumi rhodopsin (497 nm)
- Metarhodopsin I (480 nm)
- Metarhodopsin II (380 nm) 
  - (R*)
- Opsin + All-trans-retinal (380 nm)
2. Receptor 7:
Intracellular steroid receptors
Intracellular signaling protein: RAS

Cancerous mutations
- G12V, G12D, G12R
- G13V
- Q61H, Q61L

> 30% cancers contain constitutive active RAS
3. Secondary messenger 1: cAMP

Table 38-1
Hormones using cyclic AMP as a second messenger

- Calcitonin
- Chorionic gonadotropin
- Corticotropin
- Epinephrine
- Follicle-stimulating hormone
- Glucagon
- Luteinizing hormone
- Lipotropin
- Melanocyte-stimulating hormone
- Norepinephrine
- Parathyroid hormone
- Thyroid-stimulating hormone
- Vasopressin
3. **Secondary messenger 2: Ca\(^{2+}\)**

- Mediated by calcium-binding proteins such as calmodulin

- Intracellular Ca\(^{2+}\) ~ nM, inactive when increased to ~1 \(\mu\)M, Ca –complex formed

- Conformation change increases its affinity for the target proteins.

- Involved in many intracellular signalling pathways exemplified by phosphoinositide cascade and muscle contraction
3. Secondary messenger 3:
DAG and IP3

Table 38-3
Effects mediated by the phosphoinositide cascade

- Glycogenolysis in liver cells
- Histamine secretion by mast cells
- Serotonin release by blood platelets
- Aggregation of blood platelets
- Insulin secretion by pancreatic islet cells
- Epinephrine secretion by adrenal chromaffin cells
- Smooth muscle contraction
- Visual transduction in invertebrate photoreceptors

3. Secondary messenger 4:
Many others, cGMP,
4. Relevance to drug development

Disease state:
1. Abnormal level of hormones,
   Such as GABA ⇔ Epilepsy seizures, convulsions
2. Abnormal receptors
   Insulin desensitization ⇔ Type II diabetes
3. Abnormal intracellular signal transduction proteins
   Constitutive active RAS ⇔ cancers;
   Erratic tyrosine kinases ⇔ cancers and other diseases
Intervention in Receptor Signaling and Protein-Protein Interaction

Disease states:

A. Abnormal level of hormones,
   Such as GABA \( \downarrow \) Epilepsy seizures, convulsions
   Adjust hormone level by metabolic intervention
   Adjust hormone level by agonists and antagonists.

B. Abnormal receptors
   Insulin desensitization \( \Leftrightarrow \) Type II diabetes
   Mutations: usually fatal

C. Abnormal intracellular signal transduction proteins
   Constitutive active RAS \( \Leftrightarrow \) cancers;
   Erratic tyrosine kinases \( \Leftrightarrow \) cancers and other diseases
   Inhibition of kinase activities,
   break down protein-protein interaction
1. Indirect intervention of the signaling pathways:

A. Interfere in the metabolism of the hormones or messengers

Example: GABA

A negative neurotransmitter $\leftrightarrow$ glutamate (positive neurotransmitter)

GABA ↓ $\Rightarrow$ convulsion, related to epilepsy, Huntington’s disease, Parkinson’s disease, and tardive dyskinesia

Inhibit GABA-AT, GABA ↑
Inhibit GAD, GABA ↓
1. Indirect intervention of the signaling pathways:

B. Direct administration of the hormone or messenger molecules

Example 1:
Parkinson’s disease
Dopamine ↓

Example 2:
Administration of hGH to adults to increase height.

Example 3:
Use of estrogen in cosmetics.
2. Direct intervention of the signaling pathways:

**Agonist:** an agent that can evoke a maximal response like the endogeneous ligand (hormone or messenger).

**Antagonist:** an agent that binds the receptor and produce no response.

  - **Competitive:** binding the same site as the messenger.
  - **Noncompetitive:** binding a different site but affect the binding of an agonist.

**Partial agonist:** between an agonist and an antagonist, it produces a response less than the maximum.
Binding and potency of an agonist or antagonist

\[
\text{Drug + receptor} \xrightleftharpoons[k_{\text{off}}]{k_{\text{on}}} \text{drug–receptor complex}
\]

\[
K_D = \frac{[\text{drug}][\text{receptor}]}{[\text{drug–receptor complex}]}
\]

Dose-response curve for Acetylcoline (Ach) and its receptor
Response: muscle contraction
Dose-response curves

Antagonist

Competitive Antagonist

Noncompetitive Antagonist

Partial agonist

[agonist] = 0

Partial agonist

[agonist] = low

Partial agonist

[agonist] = high

[agonist] = 0
Drug-Receptor interactions

Agonist

Partial agonist or antagonist

Antagonist

Noncovalent interactions:

Drug structural features

Chirality

Geometric isomers

Conformational isomers

Ring topology

+ Van der Waals interaction
Examples of agonists and antagonists

**Agonist:**
Similar to the natural ligand.

**Antagonist:**
Resemble the natural agonist but structurally different.
Development of Analgesics from morphine
3. Intervention of protein-protein interaction

Interface at site 1: 30 AA side chains on each protein
1300 Å²
ΔG = -12.3 kcal/mol, K_D < 0.1 nM


PDB code: 3HHR
Drugs targeting signal transduction pathways

1. Very effective.

2. Many side effects because too much is involved

3. Specificity/selectivity is essential.

4. Drugs intervening protein-protein is expected to be more specific.

More reading materials:

Gadek TR. et al. ‘Generation of an LFA-1 antagonist by the transfer Of the ICAM-1 immunoregulatory epitope to a small molecule’ *Science* **2002**, 295, 1086-1089