Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. Communication allows cells to perceive and correctly respond to their microenvironment for proper development, tissue repair, and immunity and normal tissue function. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, diabetes and more. Understanding cell signaling is crucial for treating diseases effectively.

**Important functions of receptors:**
1. Globular proteins (receptors) acting as a cell’s ‘letter boxes’
2. Located mostly in the cell membrane
3. Receive messages from chemical messengers coming from other cells
4. Transmit a message into the cell leading to a cellular effect
5. Different receptors specific for different chemical messengers
6. Each cell has a range of receptors in the cell membrane making it responsive to different chemical messengers
Signals can be divided into the 5 categories below. Signal carriers (S) have to reach the proper receptors (R) for the message to be received.

**Intracrine signals** are produced by the target cell that stay within the target cell.

![Intracrine signals diagram]

**Autocrine signals** are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.

![Autocrine signals diagram]

**Juxtacrine signals** target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.

![Juxtacrine signals diagram]

**Paracrine signals** target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.

![Paracrine signals diagram]

**Endocrine signals** target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body (long-range allostery).

![Endocrine signals diagram]
Central Nervous System (CNS)

**Brain and spinal cord**
Integrative and control centers, process info out and info in

Peripheral Nervous System (PNS)

**Cranial nerves and spinal nerves**
Communication lines between the CNS and the rest of the body

Sensory (afferent) division

**Somatic and visceral sensory nerve fibers**
Conducts impulses from receptors to the CNS

Motor (efferent) division (CNS)

**Motor nerve fibers**
Conducts impulses from the CNS to effectors (muscles and glands)

Sympathetic division
mobilizes body systems during activity (fight or flight)

Autonomic nervous system (ANS)

**Visceral motor (involuntary)**
Conducts impulses from the CNS to cardiac muscles, smooth muscles, and glands

Parasympathetic division
Conserves energy
Promotes 'housekeeping' functions during rest

Somatic nervous system

**Somatic motor (voluntary)**
Conducts impulses from the CNS to skeletal muscles

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The central nervous system consists of the two major structures: the brain and spinal cord. The brain is encased in the skull, and protected by the cranium.

The spinal cord is continuous with the brain and lies on the backside to the brain, and is protected by the vertebra. The spinal cord reaches from the base of the skull, continues through or starting below the foramen magnum, and terminates roughly level with the first or second lumbar vertebra, occupying the upper sections of the vertebral canal.

The CNS integrates information it receives from, and coordinates and influences the activity of, all parts of the body and it contains the majority of the nervous system. Usually, the retina and the optic nerve (2nd cranial nerve), as well as the olfactory nerves (1st) and olfactory epithelium are considered as parts of the CNS, synapsing directly on brain tissue without intermediate ganglia.
The peripheral nervous system (PNS) is the part of the nervous system that consists of the nerves and ganglia on the outside of the brain and spinal cord. Its main function is to connect the central nervous system (CNS) to the limbs and organs, essentially serving as a communication relay going back and forth between the brain and spinal cord with the rest of the body. Unlike the CNS, the PNS is not protected by the bone of spine and skull, or by the blood–brain barrier, which leaves it exposed to toxins and mechanical injuries. The peripheral nervous system is divided into the somatic nervous system and the autonomic nervous system.
The autonomic nervous system is responsible for regulating the body's unconscious actions. The parasympathetic system is responsible for stimulation of "rest-and-digest" or "feed and breed" activities that occur when the body is at rest, especially after eating, including sexual arousal, salivation, lacrimation (tears), urination, digestion and defecation. Its action is described as being complementary to that of the sympathetic nervous system, which is responsible for stimulating activities associated with the fight-or-flight response.

**Parasympathetic nervous system**

**Sympathetic nervous system**
The somatic nervous system is the part of the peripheral nervous system associated with skeletal muscle voluntary control of body movements. It consists of afferent nerves (toward) and efferent nerves (out of). Afferent nerves are responsible for relaying sensation from the body to the central nervous system (CNS); efferent nerves are responsible for sending out commands from the CNS to the body.

There are 43 segments of nerves in the human body. With each segment, there is a pair of sensory and motor nerves. In the body, 31 segments of nerves are in the spinal cord and 12 are in the brain stem.

The somatic nervous system consists of three parts:

**Spinal nerves:** They are peripheral nerves that carry sensory information into and motor commands out of the spinal cord.

**Cranial nerves:** They are the nerve fibers that carry information into and out of the brain stem. They include smell, vision, eye, eye muscles, mouth, taste, ear, neck, shoulders, and tongue.

**Interneurons** (association nerves): These nerves integrate sensory input and motor output, numbering thousands.
Nerve cells make contact to muscle cells, organs and other nerve cells

Can be 1000s of axon inputs from other nerve cells.
Synaptic button or neuromuscular junction

NEUROSCIENCE 5e, Figure 5.3
Nerve cells and glial cells are incredibly complicated networks of connections. Perhaps, 100,000,000,000 neurons with 10,000 connections each $\sim 1,000,000,000,000,000$ (quadrillion).
Neurological disorders

Charcot–Marie–Tooth disease (CMT), also known as hereditary motor and sensory neuropathy (HMSN), hereditary sensorimotor neuropathy and peroneal muscular atrophy, is a heterogeneous inherited disorder of nerves (neuropathy) that is characterized by **loss of muscle tissue and touch sensation**, predominantly in the feet and legs but also in the hands and arms in the advanced stages of disease. Presently incurable, this disease is one of the most common inherited neurological disorders, with 37 in 100,000 affected (~1 in 2500).

Alzheimer's disease (AD), is a neurodegenerative disease characterized by **progressive cognitive deterioration** together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. The disease process is associated with plaques and tangles in the brain. The most striking early symptom is **loss of short-term memory** (amnesia), which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, cognitive (intellectual) impairment extends to the domains of language, skilled movements and recognition, and functions such as decision-making and planning become impaired.

Parkinson's disease (PD), is a degenerative **disorder of the central nervous system** that often impairs the sufferer's motor skills and speech. Parkinson's disease belongs to a group of conditions called movement disorders. It is characterized by muscle rigidity, tremor, a slowing of physical movement, and in extreme cases, a **loss of physical movement**. The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the **insufficient formation and action of dopamine**, which is produced in the dopaminergic neurons of the brain. Secondary symptoms may include high level cognitive dysfunction and subtle language problems. PD is both chronic and progressive.
Myasthenia gravis is a neuromuscular disease leading to fluctuating muscle weakness and fatigability during simple activities. Weakness is typically caused by circulating antibodies that block acetylcholine receptors at the post-synaptic neuromuscular junction, inhibiting the stimulative effect of the neurotransmitter acetylcholine.

Demyelination results in the loss of the myelin sheath insulating the nerves. When myelin degrades, conduction of signals along the nerve can be impaired or lost, and the nerve eventually withers. This leads to certain neurodegenerative disorders like multiple sclerosis, Guillain-barré syndrome and chronic inflammatory demyelinating polyneuropathy.

Axonal degeneration
Although most injury responses include a calcium influx signaling to promote resealing of severed parts, axonal injuries initially lead to acute axonal degeneration, which is rapid separation of the proximal and distal ends within 30 minutes of injury. Early changes include accumulation of mitochondria in the paranodal regions at the site of injury. Endoplasmic reticulum degrades and mitochondria swell up and eventually disintegrate. The axon undergoes complete fragmentation. The process takes about roughly 24 hrs in the PNS, and longer in the CNS.

Diabetic neuropathies are nerve damaging disorders associated with diabetes mellitus. These conditions are thought to result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum) in addition to macrovascular conditions that can culminate in diabetic neuropathy. Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. Diabetic neuropathy affects all peripheral nerves including pain fibers, motor neurons and the autonomic nervous system. Symptoms usually develop gradually over years.
cell potential = -50-89 mV because $K^+$ can diffuse out, but negatively charged proteins cannot. Sodium ions cannot move in because their ion channels are closed until triggered by a signal from the presynaptic side of the neuron. Neurotransmitters are released from the prior cell and diffuse across the synapse to bind to the ion-gate receptor and open an ion gate channel ($Na^+$ by acetyl choline, $Cl^-$ by gama aminobutyric acid and others), $Na^+$ influx depolarizes the cell down the axon and allows $Ca^{+2}$ in which causes vesicles to fuse with membrane and release the neurotransmitter, starting the next impulse.
Hormones are signaling molecules produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behaviour. Hormones have diverse chemical structures, mainly of 3 classes: **icosanoids**, **steroids**, and **amino acid derivatives** (amines, peptides, and proteins). The glands that secrete hormones comprise the **endocrine signaling system**. The term hormone is sometimes extended to include chemicals produced by cells that affect the same cell (autocrine or intracrine signalling) or nearby cells (paracrine signalling).
Liver
Insulin-like growth factor (somatomedin)
Angiotensinogen
angiotensin
Thrombopoietin

Duodenum
Secretin
Cholecystokinin

Stomach
Gastrin
Ghrelin
Peptide Y
Somatostatin
Histamine
Endothelin

Pancreas
Insulin
Glucagon
Somatostatin
Pancreatic polypeptide

Kidney
Renin
Erythropoietin
Calcitriol
Thrombopoietin

Adrenal glands
Glucocorticoids
Mineralocorticoids
Androgens

Adrenal medulla
Adrenaline
Noradrenaline
Dopamine
Enkephalin
Ovary
- Progesterone
- Androstenedione
- Estrogens
- Inhibin

Testes
- Androgens
- Estradiol
- Inhibin

Placenta (when pregnant)
- Progesterone
- Estrogens
- Human chorionic gonadotropin
- Human placental lactogen
- Inhibin

Uterus (when pregnant)
- Prolactin
- Relaxin
Calcium regulation

- Increased calcium in blood
- Calcium reabsorption from bones
- Calcium reabsorption and vitamin D hydroxylation in kidneys
- 1,25 hydroxyvitamin D
- Calcium absorption from intestines

Cholesterol -> many steps, plus sunshine -> 7-Dehydrocholesterol -> pre-vitamin D₃ -> vitamin D₃
Hormone binding to receptor - insulin

1. Insulin binds to tyrosine kinase-linked receptor.
2. Phosphorylation of receptor, starts sequence leading to synthesis of glycogen, storage of glucose (and fat too)
3. Allows influx of glucose
4. Insulin-like molecules are even found in the simplest unicellular eukaryotes, over 1 billion years old
5. Our insulin is very similar to pig and cow insulin, was used before our genes were spliced into genetically modified bacteria
**Icosanoids** are signaling molecules made by oxidation of either 20-carbon omega-3 (\( \omega-3 \)) or omega-6 (\( \omega-6 \)) fatty acids. In general, the \( \omega-6 \) eicosanoids are pro-inflammatory; \( \omega-3 \)s are much less so. There are multiple subfamilies of **eicosanoids**, including the **prostaglandins**, **thromboxanes**, and **leukotrienes**, as well as the lipoxins and eoxins, and others.

They exert complex control over many bodily systems; mainly in growth during and after physical activity, inflammation or immunity after the intake of toxic compounds and pathogens, and as messengers in the central nervous system. Many are classified as hormones. The networks of controls that depend upon eicosanoids are among the most complex in the human body.

The amounts and balance of fats in a person's diet will affect the body's eicosanoid-controlled functions, with effects on cardiovascular disease, triglycerides, blood pressure, and arthritis.

<table>
<thead>
<tr>
<th>prosta<strong>glandins</strong></th>
<th>thromboxanes</th>
<th>leukotrienes</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Prostaglandin" /></td>
<td><img src="image2.png" alt="Thromboxane" /></td>
<td><img src="image3.png" alt="Leukotriene" /></td>
</tr>
<tr>
<td><strong>alprostadil</strong> - inhibits blood clotting, vasodilator</td>
<td><strong>thromboxane A2</strong> - vasoconstrictor, hypertensive, blood clotting, aspirin inhibits its formation</td>
<td><strong>lipoxin B4</strong> - eoxin, pro-inflammatory (white blood cells), mast cells, contributes to allergies, various cancers, Hodgkin's lymphoma</td>
</tr>
</tbody>
</table>
Steroids are organic compounds with four rings arranged in a specific configuration. Examples include the dietary lipid cholesterol and the sex hormones estradiol and testosterone. Dexamethasone is an anti-inflammatory drug. Steroids have two principal biological functions: certain steroids (such as cholesterol) are important components of cell membranes which alter cell membrane fluidity, and many steroids are signaling molecules which activate steroid hormone receptors and can lead to DNA activation and protein synthesis.

Letters are used to identify the 4 rings.

glucocorticoids (adrenal cortex)
mineralocorticoids (adrenal cortex)
estrogens (gonads)
androgens (gonads)
progestins (ovaries and placenta)
vitamin D (diet and sun)
...and more
**Estradiol** is the primary female sex hormone. It is important in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is essential for the development and maintenance of female reproductive tissues but it also has important effects in many other tissues including bone. While estrogen levels in men are lower compared to women, estrogens have essential functions in men as well. It is 2 steps away from progesterone.

**Testosterone** is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. Small amounts are also secreted by the adrenal glands. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair. Levels of testosterone in adult men are about 7–8 times as great as in adult females. As the metabolic consumption of testosterone in males is greater, the daily production is about 20 times greater in men than women.
Common steroid transformations

While similar in appearance, everyone of these steroids has a specific signal to transmit, such as turning on a genetic switch. They are extremely powerful chemicals, some operating at the nanomolar level.

**5 categories of steroid hormones**

1. **progestins** - ovaries and placenta - mediate menstrual cycle and maintain pregnancy
2. **glucocorticoids** - adrenal cortex - affect metabolism in diverse ways, decrease inflammation, increase resistance to stress
3. **mineralocorticoids** - adrenal cortex - maintain salt and water balance
4. **androgens** - gonads - maturation and function of secondary sex organs, particularly in males, male sexual differentiation
5. **estrogens** - gonads - maturation and function of secondary sex organs, particularly in females

See reactions below:

- **α-estradiol** - prominent estrogen in reproductive years, more potent than estril (10x) and estrone (80x)
- **estril** - higher levels in pregnancy, marker of fetal health
- **estrone** - known female carcinogen, causes breast tenderness, in men causes anorexia
- **corticosterone** - stress hormone, immune system
- **aldosterone** - Na⁺, K⁺ balance, blood pressure reg.

Squalene, lanosterol - the compound from which all steroids are derived.

Cholesterol - essential component of cell walls, precursor of steroid hormones, bile acids and vit D

Pregnenolone - neuroactive steroid

11-deoxycorticosterone - stress

11-deoxycortisol (cortodoxone) - glucocorticoid (similar to cortisol)

Corticosterone - stress hormone, immune system

Aldosterone - Na⁺, K⁺ balance, blood pressure reg.

Statins drugs work here.

Many steps

Acetyl CoA

Common steroid transformations

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Amino acid derivatives (or from amino acids) – thyroxine, histamine, catecholamines (epinephrine, norepinephrine) oxytocin, cytokines, glutamate, glycine, gama aminobutyric acid, acetylcholine (neurotransmitters and hormones)
Chemical Messengers (deliver messages), Receptors (receive messages)

**Neurotransmitter:** A chemical released from the end of a neuron which travels across a synapse to bind with a receptor on a target cell, such as a muscle cell or another neuron. Usually short lived and responsible for messages between individual neurons.

**Hormone:** A chemical released from a cell or a gland and which travels some distance to bind with receptors on target cells throughout the body.

Chemical messengers ‘switch on’ receptors without undergoing a reaction (different from enzymes)
Structure and function of receptors

Mechanism

Receptors contain a binding site (hydrophobic hollow or cleft on the receptor protein) that is recognised by the chemical messenger

Binding of the messenger involves intermolecular bonds with amino acids

Binding results in an induced fit of the receptor protein and the messenger (alters shape)

Change in receptor shape results in a ‘domino’ effect (no reaction/catalysis, different from enzyme)

Domino effect is known as Signal Transduction, leading to a chemical signal being received inside the cell (amplification of signal is common)

Chemical messenger does not enter the cell. It departs the receptor unchanged and is not permanently bound (could find another receptor)
Binding interactions

- Ionic
- H-bonding
- van der Waals

Induced fit - Binding site alters shape to maximise intermolecular bonding

Intermolecular bonds not optimum length for maximum binding strength

Intermolecular bond lengths optimised, change of shape causes a change in function (on or off)
Overall Process of Receptor/Messenger Interaction

Signal transduction

Binding interactions must be strong enough to hold the messenger sufficiently long for signal transduction to take place

Interactions must be weak enough to allow the messenger to depart

Implies a fine balance

Designing molecules with stronger binding interactions can result in drugs that block the binding site = antagonists (agonists turn on the effect)
Receptor Superfamilies

• ION CHANNEL RECEPTORS
• G-PROTEIN COUPLED RECEPTORS
• KINASE LINKED RECEPTORS
• INTRACELLULAR RECEPTORS

MEMBRANE BOUND

RESPONSE TIME
msecs
seconds
minutes
Ion Channel Receptors

General principles

• Receptor protein is part of an ion channel protein complex

• Receptor binds a messenger leading to an induced fit

• Ion channel is opened or closed

• Ion channels are specific for specific ions (Na\(^+\), Ca\(^{2+}\), Cl\(^-\), K\(^+\))

• Ions flow across cell membrane down concentration gradient

• Polarises or depolarises nerve membranes

• Activates or deactivates enzyme-catalysed reactions within cell
Ion Channel Receptors - General principles

- Hydrophilic tunnel
- Hydrophobic exterior

Induced fit and opening of ion channel

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Ion Channel Receptors - Gating

Receptor Binding site Messenger

Cell membrane

Induced fit
‘Gating’ (ion channel opens)

Five glycoprotein subunits traversing cell membrane

Cationic ion channels for $K^+$, $Na^+$, $Ca^{2+}$ (e.g. nicotinic) = excitatory
Anionic ion channels for $Cl^-$ (e.g. $GABA_A$) = inhibitory
Ion Channel Receptors

Structure of protein subunits (4-TM receptor subunits)

4 Transmembrane (TM) regions (hydrophobic on the outside of channel)
Note: TM2 of each protein subunit ‘lines’ the central pore
(TM2 is more polar, perhaps bonded to very polar carbohydrates, TM1, TM3 and TM4 are more hydrophobic and face towards cell membrane.)
Ion Channel Receptors - Gating

• Chemical messenger binds to receptor binding site
• Induced fit results in further conformational changes
• TM2 segments rotate to open central pore

• Fast response measured in msec
• Ideal for transmission between nerves
• Binding of messenger leads directly to ion flows across cell membrane
• Ion flow can lead to secondary effects (signal transduction)
• Ion concentration within cell alters
• Leads to variation in cell chemistry
Ion Channel Receptors – Nicotinic receptor

Muscarinic agonists activate muscarinic receptors while nicotinic agonists activate nicotinic receptors. Both are direct-acting cholinomimetics that mimic acetyl choline and act on the CNS.
The glycine receptor, or GlyR, is the receptor for the amino acid neurotransmitter glycine. GlyR is an ionotropic receptor that produces its effects through chloride current. It is one of the most widely distributed inhibitory receptors in the central nervous system and has important roles in a variety of physiological processes, especially in mediating inhibitory neurotransmission in the spinal cord and brainstem.
G-Protein Coupled Receptors – General principles

- Receptor binds a messenger leading to an induced fit
- Opens a binding site for a signal protein (G-protein)
- G-Protein binds, is destabilised then split

G-Protein split into α and β/γ subunits

[Diagram showing the process of receptor binding and subsequent activation]
G-Protein Coupled Receptors – General principles

• G-Protein subunit activates membrane bound enzyme
• Binds to allosteric binding site
• Induced fit results in opening of active site
• Intracellular reaction catalysed (makes cyclic AMP, PIP₂, etc.)
G-Protein Coupled Receptors - Structure

Single protein with 7 transmembrane regions (7-TM receptor)

G protein splits into an α subunit and an β/γ subunit.
G-Protein Coupled Receptors – Ligand binding site
varies depending on receptor type

A) Monoamines: pocket in TM helices
B) Peptide hormones: top of TM helices + extracellular loops + N-terminal chain
C) Hormones: extracellular loops + N-terminal chain
D) Glutamate: N-terminal chain

Example receptors - ligands
- Monoamines - e.g. dopamine, histamine, noradrenaline, acetylcholine
- Nucleotides - e.g. Adenine, guanine, cytosine, uracil, thymine
- Lipids - e.g. Cholesterol, fatty acid, triglyceride, phospholipids
- Hormones - thyroxine, testosterone, estrogen, oxytocin, epinephrine
- Glutamate, DOPA, glycine, acetylcholine, Ca^{++}
SIGNAL TRANSDUCTION

Overview

neurotransmitter or hormone signal binds at receptor site of 7 TM receptor

There are many different types of G proteins with about 20 different types of α subunit proteins.

G protein-coupled receptors (7 TM receptors) imbedded in membranes

α subunit splits off and binds to another protein to start signal cascade inside the cell

continuing reactions inside cell

various actions inside cell
Signal Transduction involving Gs-Proteins provide one step in signal transduction pathway.

Gs-Protein - membrane bound protein of 3 subunits (α, β, γ)
- αs subunit has binding site for GDP
- GDP bound non covalently

Interaction of receptor with Gs-protein

- Process repeated for as long as ligand bound to receptor
- Signal amplification - several G-proteins activated by one ligand
- αs Subunit carries message to next stage to make cyclic AMP
Signal Transduction involving $G_s$-Proteins

Interaction of $\alpha_s$ with adenylate cyclase (inside cell)

- **Binding site for $\alpha_s$ subunit**
- **Active site (closed)**
- **Active site (open)**
- **Activation**
- **Protein Kinase A**
- **Enzyme (inactive)**
- **Enzyme (active)**
- **Enzyme-catalysed reaction**
- **$\alpha_s$ Subunit changes shape**
- **Weaker binding to enzyme**
- **Departure of subunit**
- **Enzyme reverts to inactive state**
- **$\alpha_s$ Subunit recombines with $\beta,\gamma$ dimer to reform $G_s$ protein**
Signal Transduction involving $G_s$-Proteins

Interaction of cyclic AMP with protein kinase A (PKA)

**Protein kinase A** - 4 protein subunits
- 2 regulatory subunits (R) and 2 catalytic subunits (C)

- Cyclic AMP binds to PKA
- Induced fit destabilises complex
- Catalytic units released and activated

Phosphorylation of other proteins and enzymes
Signal continued by phosphorylated proteins
Further signal amplification

$\text{Protein} + \text{ATP}$

$\text{Protein} + \text{ADP}$
Signal Transduction involving Gs-Proteins

Glycogen Metabolism - triggered by adrenaline in liver cells

Adrenaline $\rightarrow$ signal received $\rightarrow$ $\alpha_s$ $\rightarrow$ adenylate cyclase $\rightarrow$ cAMP $\rightarrow$ Protein kinase A $\rightarrow$ Catalytic subunit of PKA $\rightarrow$ Inhibitor (inactive) $\rightarrow$ Phosphatase (inhibited)

Glycogen synthase (active) $\rightarrow$ Inhibitor-P (active) $\rightarrow$ Phosphorylase kinase (inactive) $\rightarrow$ Phosphorylase kinase P (active) $\rightarrow$ Phosphorylase b (inactive) $\rightarrow$ Phosphorylase b (active) $\rightarrow$ Glycogen $\rightarrow$ Glucose-1-phosphate

β-Adrenoreceptor
Signal Transduction involving $G_s$-Proteins

Interaction of $\alpha_s$ with adenylate cyclase converts ATP to cAMP

- Several 100 ATP molecules converted before $\alpha_s$-GTP deactivated
- Represents another signal amplification
- Cyclic AMP becomes next messenger (secondary messenger)
- Cyclic AMP enters cell cytoplasm with message (possible further amplification)
Signal Transduction involving $G_\beta$-Proteins

Glycogen Metabolism - triggered by adrenaline in liver cells

Coordinated effect: activation of glycogen metabolism ($\rightarrow$ glucose $\rightarrow$ glycolysis $\rightarrow$ energy)
inhibition of glycogen synthesis (no gluconeogenesis)

Adrenaline has different effects on different cells
e.g. activates fat metabolism in fat cells ($\rightarrow$ acetyl CoA $\rightarrow$ glucose = slower)

Drugs interacting with cyclic AMP signal transduction
Cholera toxin causes constant activation of cyclic AMP leading to diahorrea

Theophylline and caffeine
- inhibit phosphodiesterases
- phosphodiesterases responsible for metabolizing cyclic AMP
- cyclic AMP activity is prolonged (keeps you wired)

\[ \begin{align*}
\text{Theophylline} & : & \text{Caffeine} \\
\text{H}_3\text{C} & - & \text{CH}_3 \\
\text{N} & - & \text{N} \\
\text{O} & - & \text{O} \\
\text{H} & - & \text{CH}_3 \\
\text{N} & - & \text{N} \\
\text{O} & - & \text{O} \\
\text{CH}_3 & - & \text{H}_3\text{C}
\end{align*} \]
**Signal Transduction involving Gs-Proteins**

**Interaction of cyclic AMP with protein kinase A (PKA)**

**Protein kinase A is a serine-threonine kinase**

**Activated by cyclic AMP**

**Catalyses phosphorylation of serine and threonine residues on protein substrates**

**Phosphate unit provided by ATP**

Protein kinases modify other proteins by chemically adding phosphate groups to them (phosphorylation). This usually results in a functional change of the target protein. The human genome contains about 500 protein kinase genes and they constitute about 2% of all human genes. Up to 30% of all human proteins may be modified by kinase activity, and kinases are known to regulate the majority of cellular pathways, especially those involved in signal transduction.
G-Protein Coupled Receptors – bacteriorhodopsin / rhodopsin family

- **Rhodopsin** = visual receptor
- Many common receptors belong to this same family (isozymes)
- Implications for drug selectivity depending on receptor similarity – consequence of evolution
- Membrane bound receptors difficult to crystallise
- **X-Ray structure of bacteriorhodopsin solved** - bacterial protein similar to rhodopsin
- Bacteriorhodopsin structure used as ‘template’ for other receptors
- Construct model receptors based on template and amino acid sequence
- Leads to model binding sites for drug design
- **Crystal structures for rhodopsin and β₂-adrenergic receptors now solved** - better templates
Ethene has a pi bond. Pi bonds are second and third bonds and are generally weaker. MO diagram of a pi bond.

LUMO = lowest unoccupied molecular orbital
HOMO = highest occupied molecular orbital

Ethene MO diagram

\[ \pi_{CC} = 2p_a - 2p_b = \text{antibonding MO} \]
\[ \pi_{CC} = 2p_a + 2p_b = \text{bonding MO} \]

energy of starting atomic orbitals

\[ \sigma_{CC} = sp^2_a - sp^2_b = \text{antibonding MO} \]
\[ \sigma_{CC} = sp^2_a + sp^2_b = \text{bonding MO} \]

Total bonding e's = 12 e's

\[ 2C = 8 \text{ e's} \]
\[ 4H = 4 \text{ e's} \]

Bond order = \( \frac{(12) - (0)}{2} = 6 \) bonds
buta-1,3-diene - conjugated pi bonds

More nodes is less stable = no electron density between adjacent atoms.

\[ \psi_4 = \pi_1^* - \pi_2^* \]

\[ \psi_3 = \pi_1^* + \pi_2^* = \text{LUMO} \]

\[ \psi_2 = \pi_1 - \pi_2 = \text{HOMO} \]

\[ \psi_1 = \pi_1 + \pi_2 \]

Energy gap between HOMO and LUMO orbitals in pi bond versus diene.

\[ \Delta E = \hbar \nu = \hbar c (1/\lambda) \]

\[ \hbar = \text{Plank's constant} = 6.62 \times 10^{-34} \text{ J-sec} \]

\[ c = 3.0 \times 10^8 \text{ m/sec} \]

\[ \nu = \text{frequency (#/sec = Hz)} \]

\[ \lambda = \text{wave length (nm = } 10^{-9} \text{m)} \]

These are variables that indicate the energy being measured. Only one of them is needed to specify the energy.
The HOMO/LUMO energy gap can be measured with light (frequency = \( \nu \) or wave length = \( \lambda \))

\[ \lambda_{\text{max}} \] (wave length measure of HOMO/LUMO energy gap)

<table>
<thead>
<tr>
<th>( \lambda ) (nm)</th>
<th>( \Delta E ) (HOMO/LUMO energy gap)</th>
</tr>
</thead>
<tbody>
<tr>
<td>174</td>
<td>larger ( \Delta E )</td>
</tr>
<tr>
<td>217</td>
<td>smaller ( \lambda ), indicates a larger ( \Delta E )</td>
</tr>
<tr>
<td>250</td>
<td>*</td>
</tr>
<tr>
<td>280</td>
<td>*</td>
</tr>
<tr>
<td>310</td>
<td>*</td>
</tr>
<tr>
<td>340</td>
<td>larger ( \lambda ), indicates a smaller ( \Delta E )</td>
</tr>
<tr>
<td>370</td>
<td>*</td>
</tr>
<tr>
<td>400</td>
<td>smaller ( \Delta E ) (HOMO/LUMO energy gap)</td>
</tr>
</tbody>
</table>

\[ \Delta E = (hc) \left( \frac{1}{\lambda} \right) \]

\* = estimated value

UV is higher energy electromagnetic radiation that can damage chemical bonds

IR is lower energy electromagnetic radiation that we sense as heat

Increasing Energy

\( \beta \)-carotene (this is the most common structure given, but there are hundreds of variations known in nature)

lycopene (found in tomatoes and other vegetables, also has hundreds of variations known in nature)
Increasing Energy

absorbs visible light (depends on the protein charge and twist in conformation), and uses the energy to break the cis pi bond, isomerizing cis to trans, which causes a release of 11-trans-retinal from the protein and starts a cascade of reactions (cell polarization) leading to "vision".

The release of retinal causes polarization of the cell membrane, ion channels open up and Na+ rushes in and K+ rushes out and an impulse travels to the synapse where neurotransmitters are released and complex at receptors of the next cell (neuron) which causes that cell to polarize and continue the signal to the visual part of your brain where it constructs an image that you see.
How does vision work?
Slightly different protein environments, changes HOMO/LUMO gap and what part of visible region gets absorbed. Your genetics determines your protein structure and the colors that you see. Different variations of protein complexes (twisting and charge distribution) absorb different wavelengths of light.

$$\Delta E = h \nu = h c \left( \frac{1}{\lambda} \right)$$

What would happen if one of these was defective?

R = red  
O = orange  
Y = yellow  
G = green  
B = blue  
I = indigo  
V = violet

Rhodopsin complex imbedded in cell membrane has retinal bound perpendicular to 7 transmembrane protein helicies. Isomerization triggers cell action potential (polarization) and Na+ flows in and K+ flows out (1 millisec). In some cells this is followed by Ca+2 ions efflux (100 millisec).

At a synapse, neurotransmitters are released from vesicles, triggering the next neuron to fire and transmit the signal to the appropriate parts of the brain.

AC = acetylcholine used in peripheral and central nervous systems

GLU = glutamate / glutamic acid important neurotransmitter in the brain and spinal cord

DPA = dopamine used in several parts of the brain, cocaine and methamphetamine act on dopamine receptors, Parkinson's is caused by loss of dopamine secretion
G-Protein Coupled Receptors – bacteriorhodopsin / rhodopsin family

Common ancestor

divergent evolution

Monoamines

Opsins, Rhodopsins

Convergent evolution

Convergent evolution

Endothelins

Bradykinin

Angiotensin

Interleukin-8

Tachkinins

Muscarinic

Histamine

α-Adrenergic

Dopaminergic

β-Adrenergic

Receptor types

Receptor subtypes
G-Protein Coupled Receptors – Receptor types and subtypes

Reflects differences in receptors which recognise the same ligand

<table>
<thead>
<tr>
<th>Receptor Types</th>
<th>Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic Alpha (α)</td>
<td>α₁, α₂A, α₂B, α₂C</td>
</tr>
<tr>
<td>Adrenergic Beta (β)</td>
<td>β₁, β₂, β₃</td>
</tr>
<tr>
<td>Cholenergic Nicotinic</td>
<td>M₁–M₅</td>
</tr>
<tr>
<td>Cholenergic Muscarinic</td>
<td></td>
</tr>
</tbody>
</table>

• Receptor types and subtypes are not equally distributed amongst tissues
• Target selectivity leads to tissue selectivity

Heart muscle β₁ adrenergic receptors
Fat cells β₃ adrenergic receptors
Bronchial muscle α₁ & β₂ adrenergic receptors
GI-tract α₁, α₂ & β₂ adrenergic receptors
Signal Transduction involving $G_i$-Proteins ($i = \text{inhibition}$)

- Bind to different receptors from those used by $G_s$ proteins
- **Mechanism of activation is identical**
- $\alpha_i$ subunit binds to adenylate cyclase and inhibits it
- **Adenylate cyclase is under dual control (brake/accelerator)**
- Background activity due to constant levels of $\alpha_s$ and $\alpha_i$
- **Overall effect depends on dominant alpha subunit ($\alpha_s$ or $\alpha_i$)**
- Dominant alpha subunit depends on receptors activated
Phosphorylation Reactions

• Prevalent in activation and deactivation of enzymes
• Phosphorylation radically alters intramolecular binding
• Results in altered conformations

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Phosphorylation Reactions

• Prevalent in activation and deactivation of enzymes
• Phosphorylation radically alters intramolecular binding
• Results in altered conformations
Signal Transduction involving G_q Proteins

Interaction with phospholipase C (PLC)

- G_q proteins - interact with different receptors from those recognised by G_S and G_I
- Split by the same mechanism to give an α_q subunit
- The α_q subunit activates or deactivates PLC (membrane bound enzyme)
- Reaction catalysed for as long as α_q bound - signal amplification
- Brake and accelerator effect

GTP hydrolysis

Binding weakened
Signal Transduction involving $G_q$ Proteins

Interaction with phospholipase C (PLC)

Phosphatidylinositol diphosphate (integral part of cell membrane)

Inositol triphosphate (polar and moves into cell cytoplasm)

Diacylglycerol (remains in membrane)

$R = \text{long chain hydrocarbons} \quad \text{and} \quad \overset{\ldots}{P} = \text{PO}_3^{2-}$

$\text{PIP}_2$ phosphatidylinositol diphosphate

$\text{IP}_3$ inositol triphosphate

$\text{DG}$ diacylglycerol
Signal Transduction involving $G_q$ Proteins

Action of diacylglycerol

- Activates protein kinase C (PKC)
- PKC moves from cytoplasm to membrane
- Phosphorylates Ser & Thr residues of protein substrates
- Activates enzymes to catalyse intracellular reactions
- Linked to inflammation, tumour propagation, smooth muscle activity etc
Signal Transduction involving $G_q$ Proteins

Resynthesis of PIP$_2$ – phosphatidylinositol diphosphate

\[
\begin{align*}
\text{IP}_3 & \xrightarrow{\text{phosphatase}} \text{IP}_2 & \xrightarrow{\text{phosphatase}} \text{IP} & \xrightarrow{\text{phosphatase}} \text{inositol} & \xrightarrow{\text{PI synthase}} \text{PI} & \xrightarrow{\text{PI-4-kinase}} \text{PIP}_3 & \xrightarrow{\text{PI-4-P-5-kinase}} \text{PIP}_2
\end{align*}
\]

Li$^+$ salts for manic depressive illness

IP$_3$ → IP$_2$ → IP → inositol → PI → PIP$_3$ → PIP$_2$

DG = diacylglycerol
Signal Transduction involving $G_q$ Proteins

Action of diacylglycerol

Drugs inhibiting Protein Kinase C (PKC) - potential anti cancer agents

Bryostatin (from sea moss)

Text, Fig 21.70 p. 566
Signal Transduction involving $G_q$ Proteins

Action of inositol triphosphate

- $IP_3$ is hydrophilic and enters the cell cytoplasm
- Mobilizes $Ca^{2+}$ release in cells by opening $Ca^{2+}$ ion channels
- $Ca^{2+}$ activates protein kinases
- Protein kinases activate intracellular enzymes
- Cell chemistry is altered leading to a biological effect

Calmodulin = calcium modulated protein, interacts with kinases and phosphatases

Cell membrane

<table>
<thead>
<tr>
<th>Calcium stores</th>
<th>$IP_3$</th>
<th>$Ca^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cytoplasm

<table>
<thead>
<tr>
<th>Calmodulin</th>
<th>$Ca^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Protein kinase</th>
<th>Enzyme</th>
<th>Enzyme (inactive)</th>
<th>Enzyme (active)</th>
<th>Enzyme-catalysed reaction</th>
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Tyrosine Kinase Linked Receptors

- Bifunctional receptor / enzyme
- Activated by hormones
- Protein serves dual role - receptor plus enzyme
- Receptor binds messenger leading to an induced fit
- Protein changes shape and opens intracellular active site
- Reaction catalysed within cell
- Overexpression related to several cancers

Diagram:
- Messenger binds to closed receptor
- Induced fit opens active site
- Intracellular reaction occurs
- Overexpression related to several cancers
Tyrosine Kinase Linked Receptors - Structure

Extracellular
N-terminal chain

Ligand binding region

Hydrophilic transmembrane region (α-helix)

Cell membrane

Catalytic binding region (closed in resting state)

Intracellular C-terminal chain in cytosol

Tyrosine kinase
Tyrosine Kinase Linked Receptors – reaction catalyzed by tyrosine kinase

![Diagram of tyrosine kinase reaction](image-url)

- Tyrosine kinase catalyzes the reaction.
- Mg$^{2+}$ is involved in the reaction.
- ATP is the energized substrate, and ADP is the product.
- Tyrosine amino acid is the substrate, and phosphorylated tyrosine residue is the product.
**Tyrosine Kinase Linked Receptors – Epidermal growth factor receptor (EGF-R)**

- **Binding site for EGF**
- **EGF - protein hormone - bivalent ligand**
- **Active site of tyrosine kinase**

Active site on one half of dimer catalyses phosphorylation of Tyr residues on other half

- **Dimerisation of receptor is crucial**
- Phosphorylated regions act as binding sites for further proteins and enzymes
- **Results in activation of signalling proteins and enzymes**
- **Message carried into cell**
Tyrosine Kinase Linked Receptors Insulin receptor (tetrameric complex)

- Insulin binding site
- Kinase active site

Insulin binding activates the receptor, opening the kinase active site through induced fit.

Phosphorylation occurs, with ATP being converted to ADP and 

\[
\text{ATP} \rightarrow \text{ADP} + \text{PO}
\]
Tyrosine Kinase Linked Receptors - Growth hormone receptor
Tetrameric complex constructed in presence of growth hormone

- **GH binding & dimerisation**

- **Binding of kinases**

- **Activation and phosphorylation**

  - ATP → ADP

  - Kinase active site opened by induced fit

- **Growth hormone binding site**

- **Kinase active site**
Signal Transduction - Tyrosine Kinase Linked Receptors

Signalling pathways

1-TM Receptors

Tyrosine kinase inherent or associated

Signalling proteins

PLCγ

IP3 kinase

IP3

DG

Ca++

PKC

IP3

DG

PIP3

GAP

Grb2

Others

Guanylate cyclase

cGMP

cyclic GMP

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Signal Transduction - Tyrosine Kinase Linked Receptors

Example of a signalling pathway

1) Binding of growth factor

2) Conformational change

Growth factor

1) Binding of growth factor

2) Conformational change

Phosphorylation

Grb2

Binding and phosphorylation of Grb2

Binding Ras and GTP/GDP exchange

involved in cell growth, differentiation and survival
Signal Transduction - Tyrosine Kinase Linked Receptors

Example of a signalling pathway

1) Binding of growth factor
2) Conformational change

Dimerisation

Phosphorylation

Growth factor

Binding and phosphorylation of Grb2

GTP/GDP exchange

Binding Ras and

GDP

GTP

involved in cell growth, differentiation and survival
Signal Transduction - Tyrosine Kinase Linked Receptors

Example of a signalling pathway

Transcription factor (inactive) → Transcription factor (active)

Map kinase (inactive) → Map kinase (active)

Mek (inactive) → Mek (active)

Raf (inactive) → Raf (active)

Gene transcription
Intracellular Receptors - Structure

- Chemical messengers must cross cell membrane
- Chemical messengers must be hydrophobic
- Example - steroids and steroid receptors

Zinc fingers contain Cys residues (SH)
Allow S-Zn interactions with proteins that bind to DNA and RNA
Intracellular Receptors – Mechanism

1. Messenger crosses membrane
2. Binds to receptor
3. Receptor dimerisation
4. Binds co-activator protein
5. Complex binds to DNA
6. Transcription switched on or off
7. Protein synthesis activated or inhibited
Intracellular Receptors – Estrogen receptor

- Estradiol binds to the estrogen receptor, leading to dimerisation and exposure of AF-2 regions.
- This exposes the AF-2 regions, allowing coactivators to bind.
- Coactivators enhance transcription factor activity, leading to transcription.

Chemical structures:
- Testosterone: \( \text{CH}_3 \text{CH}_2\text{CH} = \text{CH} - \text{CH} - \text{CH}_2\text{OH} \)
- Estradiol / Estrogen: \( \text{HO} - \text{CH}_3\text{CH} = \text{CH} - \text{CH} - \text{CH}_2\text{OH} \)
The oxidation of testosterone to make estradiol shows yet another way of losing a methyl group through a conjugated C=C bond.
Overview of signal transduction pathways
**Signal transduction** occurs when an extracellular signaling molecule activates a specific receptor located on the cell surface or inside the cell. That receptor triggers a biochemical chain of events inside the cell. Depending on the cell, the response alters the cell's metabolism, shape, gene expression, ability to divide and more. The signal can be amplified at any step.

**G protein-coupled receptors** integral transmembrane proteins that possess seven transmembrane domains and are linked to a heterotrimeric G protein. (includes adrenergic receptors and chemokine receptors.)

**Tyrosine kinase receptors** are transmembrane proteins with an intracellular kinase domain and an extracellular domain that binds ligands; (includes growth factor receptors such as the insulin receptor)

**Integrins** play a role in cell attachment to other cells and the extracellular matrix and in the transduction of signals from extracellular matrix components such as fibronectin and collagen.

**Toll-like receptors** (TLRs) take adapter molecules within the cytoplasm of cells in order to propagate a signal. Thousands of genes are activated by TLR signaling, implying that this method constitutes an important gateway for gene modulation.

A **ligand-gated ion channel**, upon binding with a ligand, changes conformation to open a channel in the cell membrane through which ions relaying signals can pass. An example of this mechanism is found in the receiving cell of a neural synapse.

**Intracellular receptors**, such as nuclear receptors and cytoplasmic receptors, are soluble proteins localized within their respective areas. The typical ligands for nuclear receptors are non-polar hormones like the steroid hormones testosterone and progesterone and derivatives of vitamins A and D. To initiate signal transduction, the ligand must pass through the plasma membrane by passive diffusion. On binding with the receptor, the ligands pass through the nuclear membrane into the nucleus, altering gene expression. Nucleic receptors have DNA-binding domains containing zinc fingers and a ligand-binding domain; the zinc fingers stabilize DNA binding by holding its phosphate backbone.

**Second messengers**: Ca\(^{+2}\), lipophilics (diacylglycerol), nitric oxide and other redox signaling molecules