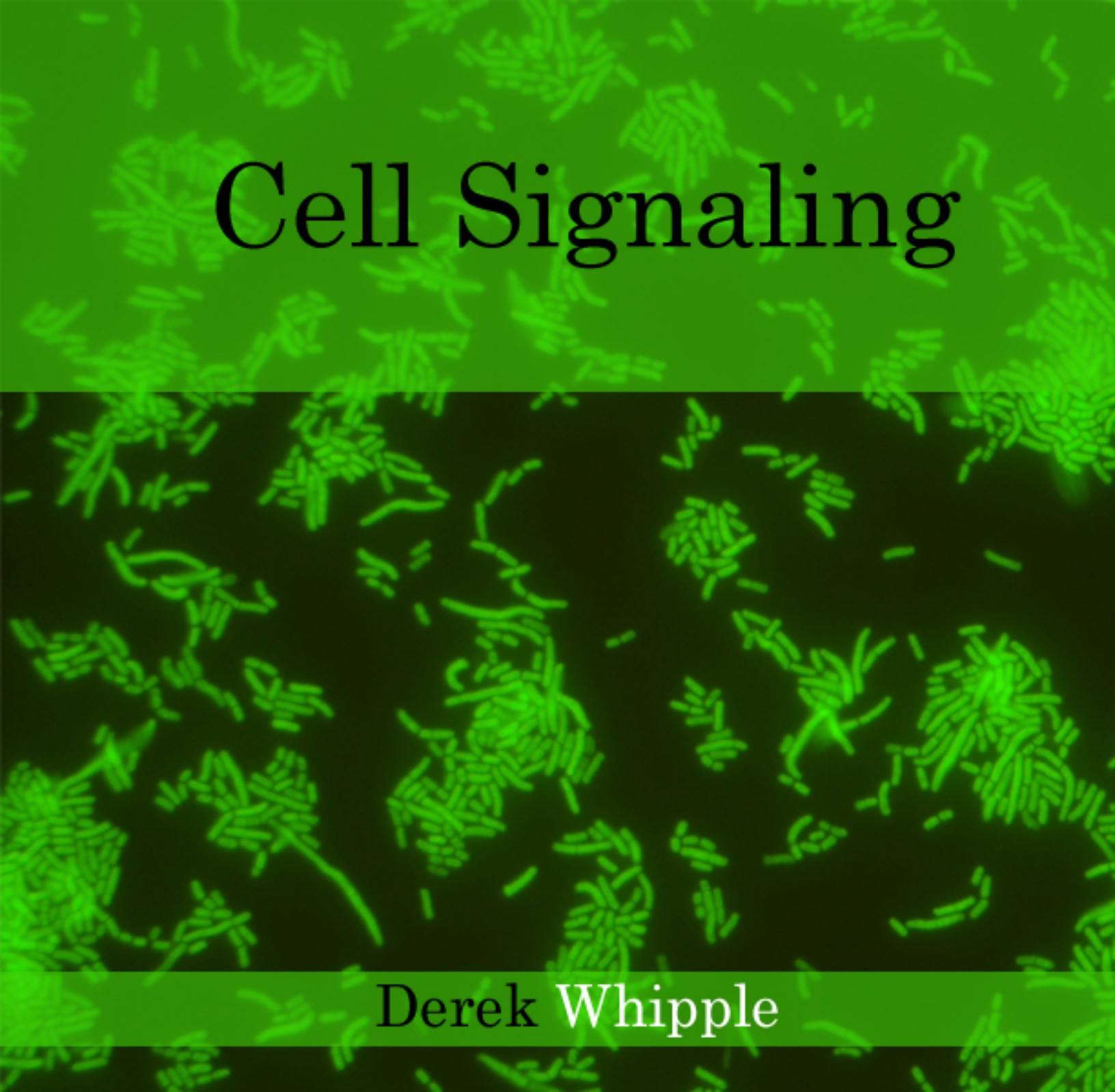
Cell Signaling



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Cell Signaling

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be created.

Traditional work in biology has focused on studying individual parts of cell signaling pathways. Systems biology research helps us to understand the underlying structure of cell signaling networks and how changes in these networks may affect the transmission and flow of information. Such networks are complex systems in their organization and may exhibit a number of emergent properties including bistability and ultrasensitivity. Analysis of cell signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of simulations and modelling.

Unicellular and multicellular organism cell signaling

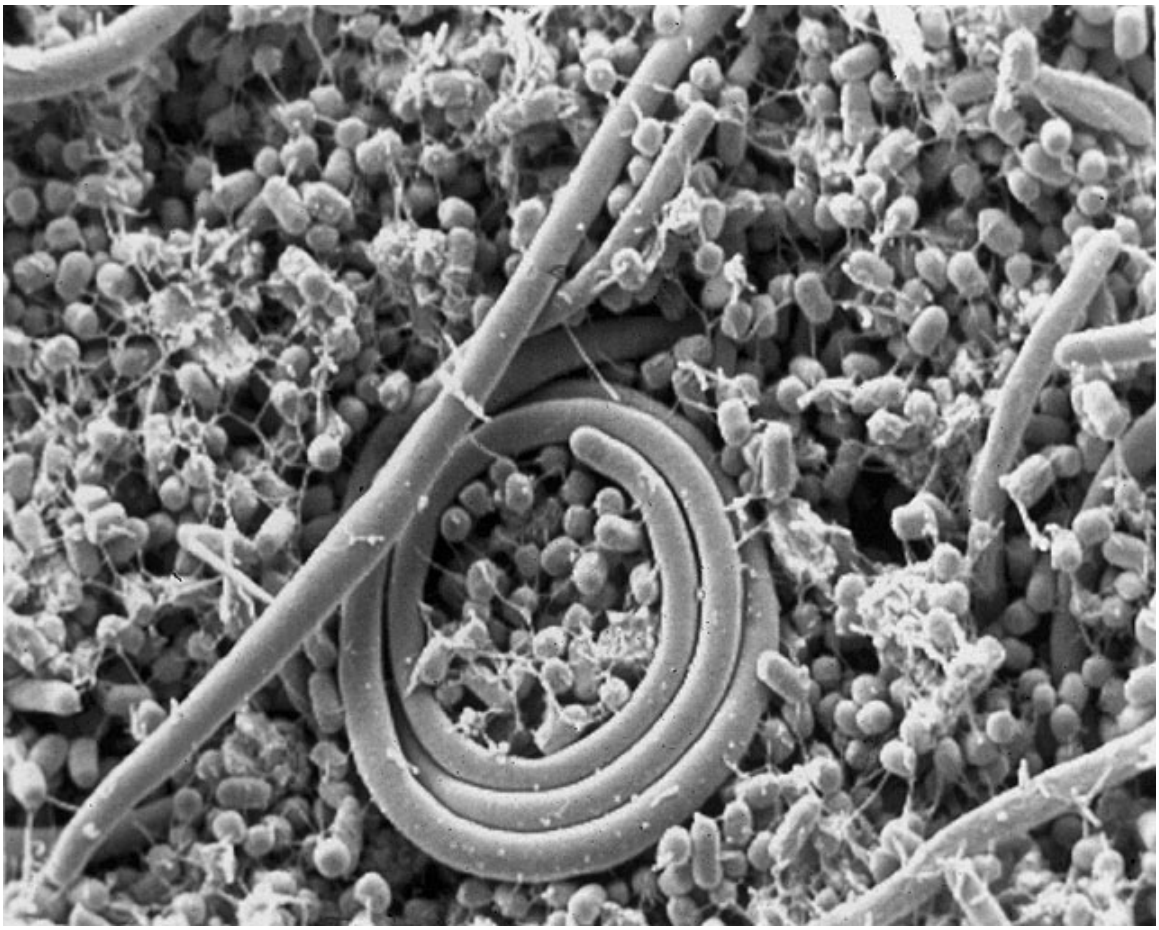


Figure 1. Example of signaling between bacteria. Salmonella enteritidis uses acyl-homoserine lactone for Quorum sensing (see: Inter-Bacterial Communication)

Cell signaling has been most extensively studied in the context of human diseases and signaling between cells of a single organism. However, cell signaling may also occur between the cells of two different organisms. In many mammals, early embryo cells exchange signals with cells of the uterus. In the human gastrointestinal tract, bacteria exchange signals with each other and with human epithelial and immune system cells. For the yeast *Saccharomyces cerevisiae* during mating, some cells send a peptide signal (mating factor *pheromones*) into their environment. The mating factor peptide may bind to a cell surface receptor on other yeast cells and induce them to prepare for mating.

Types of signals

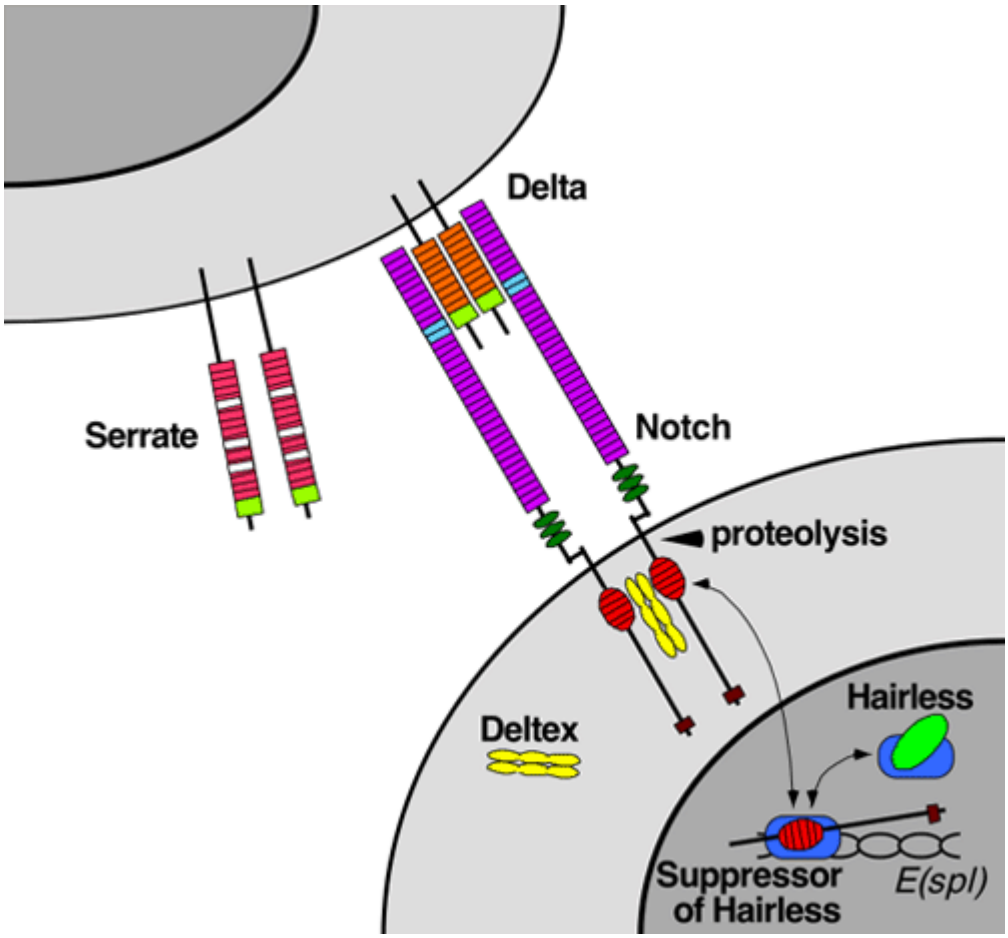


Figure 2. Notch-mediated juxtacrine signal between adjacent cells.

Cells communicate with each other via direct contact (juxtacrine signaling), over short distances (paracrine signaling), or over large distances and/or scales (endocrine signaling).

Some cell-to-cell communication requires direct cell-cell contact. Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. In cardiac muscle, gap junctions between adjacent cells allows for action potential propagation from the cardiac pacemaker region of the heart to spread and coordinately cause contraction of the heart.

The Notch signaling mechanism is an example of juxtacrine signalling (also known as contact-dependent signaling) in which two adjacent cells must make physical contact in order to communicate. This requirement for direct contact allows for very precise control of cell differentiation during embryonic development. In the worm *Caenorhabditis elegans*, two cells of the developing gonad each have an equal chance of terminally differentiating or becoming a uterine precursor cell that continues to divide. The choice of which cell continues to divide is controlled by competition of cell surface signals. One

cell will happen to produce more of a cell surface protein that activates the Notch receptor on the adjacent cell. This activates a feedback loop or system that reduces Notch expression in the cell that will differentiate and that increases Notch on the surface of the cell that continues as a stem cell.

Many cell signals are carried by molecules that are released by one cell and move to make contact with another cell. *Endocrine* signals are called hormones. Hormones are produced by endocrine cells and they travel through the blood to reach all parts of the body. Specificity of signaling can be controlled if only some cells can respond to a particular hormone. *Paracrine* signals such as retinoic acid target only cells in the vicinity of the emitting cell. Neurotransmitters represent another example of a paracrine signal. Some signaling molecules can function as both a hormone and a neurotransmitter. For example, epinephrine and norepinephrine can function as hormones when released from the adrenal gland and are transported to the heart by way of the blood stream. Norepinephrine can also be produced by neurons to function as a neurotransmitter within the brain. Estrogen can be released by the ovary and function as a hormone or act locally via paracrine or autocrine signaling. Active species of oxygen and nitric oxide can also act as cellular messengers. This process is dubbed redox signaling.

Receptors for cell moves

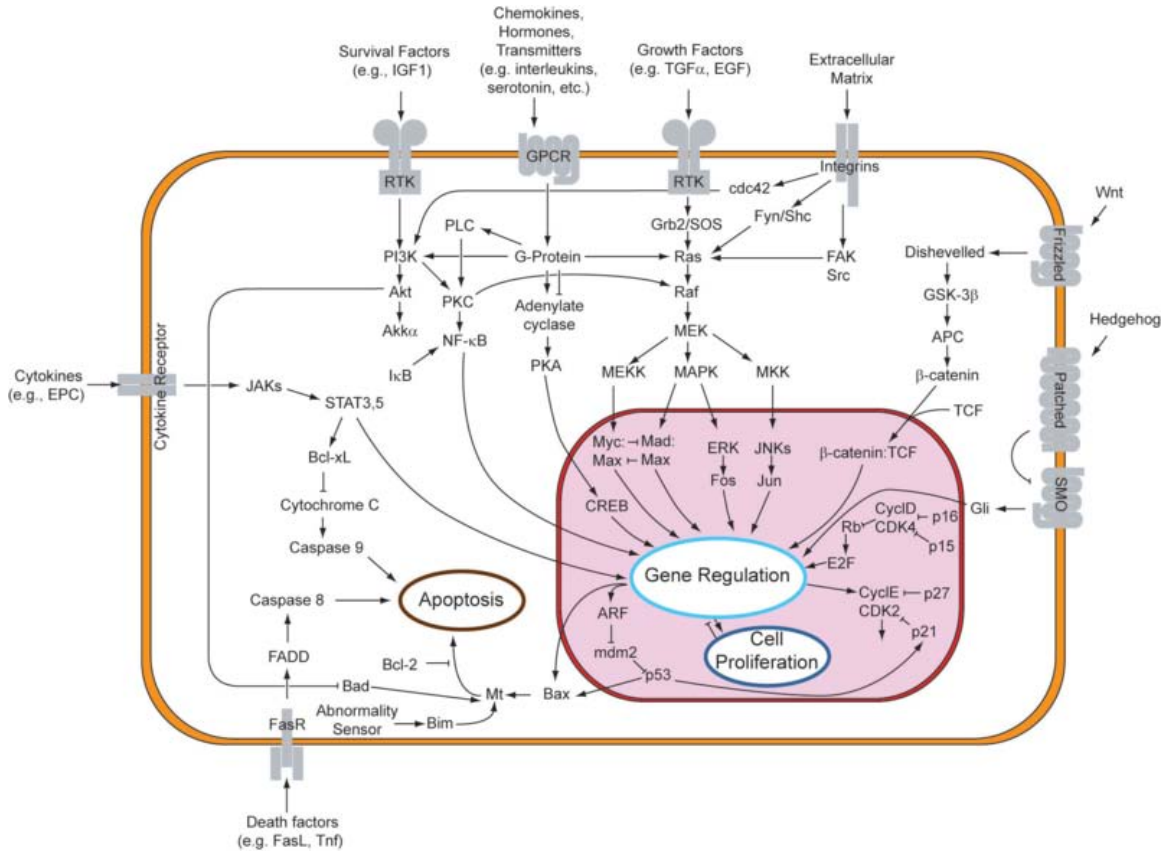
Cells receive information from their environment through a class of proteins known as receptors. Notch is a cell surface protein that functions as a receptor. Animals have a small set of genes that code for signaling proteins that interact specifically with Notch receptors and stimulate a response in cells that express Notch on their surface. Molecules that activate (or, in some cases, inhibit) receptors can be classified as hormones, neurotransmitters, cytokines, growth factors but all of these are called receptor ligands. The details of ligand-receptor interactions are fundamental to cell signaling.

As shown in Figure 2 (above, left), Notch acts as a receptor for ligands that are expressed on adjacent cells. While many receptors are cell surface proteins, some are found inside cells. For example, oestrogen is a hydrophobic molecule that can pass through the lipid bilayer of cell surface membranes. Oestrogen receptors inside cells of the uterus can be activated by oestrogen that comes from the ovaries, enters the target cells, and binds to oestrogen receptors.

A number of transmembrane receptors for molecules that include peptide hormones and of intracellular receptors for steroid hormones exist, giving to a cell the ability to respond to a great number of hormonal and pharmacological stimuli. In diseases, often, proteins that interact with receptors are aberrantly activated, resulting in constitutively activated downstream signals.

For several types of intercellular signaling molecules that are unable to permeate the hydrophobic cell membrane due to their hydrophilic nature, the target receptor is expressed on the membrane. When such signaling molecule activates its receptor, the signal is carried into the cell usually by means of a second messenger such as cAMP.

Signaling pathways



Overview of signal transduction pathways.

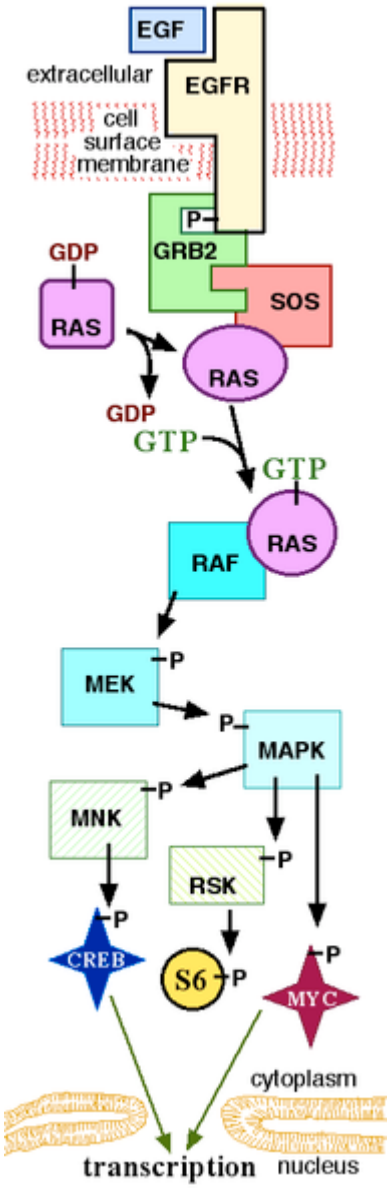


Figure 3. Diagram showing key components of a signal transduction pathway.

In some cases, receptor activation caused by ligand binding to a receptor is directly coupled to the cell's response to the ligand. For example, the neurotransmitter GABA can activate a cell surface receptor that is part of an ion channel. GABA binding to a GABA A receptor on a neuron opens a chloride-selective ion channel that is part of the receptor. GABA A receptor activation allows negatively-charged chloride ions to move into the neuron, which inhibits the ability of the neuron to produce action potentials. However, for many cell surface receptors, ligand-receptor interactions are not directly linked to the cell's response. The activated receptor must first interact with other proteins inside the cell before the ultimate physiological effect of the ligand on the cell's behavior is produced. Often, the behavior of a chain of several interacting cell proteins is altered

following receptor activation. The entire set of cell changes induced by receptor activation is called a signal transduction mechanism or pathway.

In the case of Notch-mediated signaling, the signal transduction mechanism can be relatively simple. As shown in Figure 2 (above, left), activation of Notch can cause the Notch protein to be altered by a protease. Part of the Notch protein is released from the cell surface membrane and can act to change the pattern of gene transcription in the cell nucleus. This causes the responding cell to make different proteins, resulting in an altered pattern of cell behavior. Cell signaling research involves studying the spatial and temporal dynamics of both receptors and the components of signaling pathways that are activated by receptors in various cell types.

A more complex signal transduction pathway is shown in Figure 3. This pathway involves changes of protein-protein interactions inside the cell, induced by an external signal. Many growth factors bind to receptors at the cell surface and stimulate cells to progress through the cell cycle and divide. Several of these receptors are kinases that start to phosphorylate themselves and other proteins when binding to a ligand. This phosphorylation can generate a binding site for a different protein and thus induce protein-protein interaction. In Figure 3, the ligand (called epidermal growth factor (EGF)) binds to the receptor (called EGFR). This activates the receptor to phosphorylate itself. The phosphorylated receptor binds to an adaptor protein (GRB2), which couples the signal to further downstream signaling processes. For example, one of the signal transduction pathways that are activated is called the mitogen-activated protein kinase (MAPK) pathway. The signal transduction component labeled as "MAPK" in the pathway was originally called "ERK," so the pathway is called the MAPK/ERK pathway. The MAPK protein is an enzyme, a protein kinase that can attach phosphate to target proteins such as the transcription factor MYC and, thus, alter gene transcription and, ultimately, cell cycle progression. Many cellular proteins are activated downstream of the growth factor receptors (such as EGFR) that initiate this signal transduction pathway.

Some signaling transduction pathways respond differently depending on the amount of signaling received by the cell. For instance, the hedgehog protein activates different genes, depending on the amount of hedgehog protein present.

Complex multi-component signal transduction pathways provide opportunities for feedback, signal amplification, and interactions inside one cell between multiple signals and signaling pathways.

Classification of intercellular communication

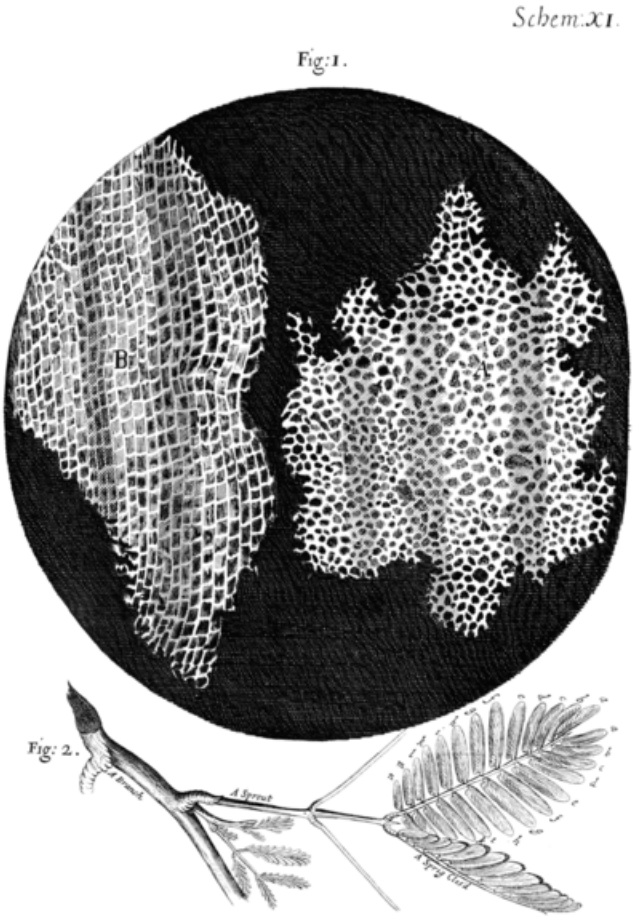
Within endocrinology (the study of intercellular signalling in animals) and the endocrine system, intercellular signalling is subdivided into the following classifications:

- *Intracrine* signals are produced within the target cell.

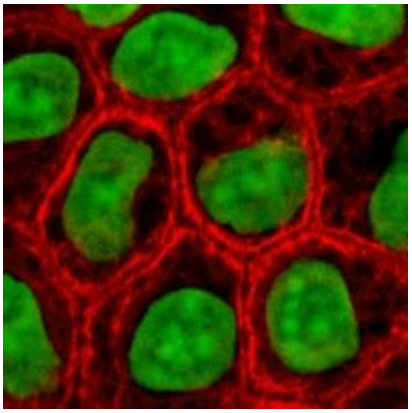
- *Autocrine* signals target the cell itself. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- *Juxtacrine* signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- *Paracrine* signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- *Endocrine* signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

Chapter- 2

Cell (Biology)



Drawing of the structure of cork as it appeared under the microscope to Robert Hooke from Micrographia which is the origin of the word "**cell**" being used to describe the smallest unit of a living organism



Cells in culture, stained for keratin (red) and DNA (green)

The **cell** is the functional basic unit of life. It was discovered by Robert Hooke and is the functional unit of all known living organisms. It is the smallest unit of life that is classified as a living thing, and is often called the building block of life. Some organisms, such as most bacteria, are unicellular (consist of a single cell). Other organisms, such as humans, are multicellular. Humans have about 100 trillion or 10^{14} cells; a typical cell size is 10 µm and a typical cell mass is 1 nanogram. The largest cells are about 135 µm in the anterior horn in the spinal cord while granule cells in the cerebellum, the smallest, can be some 4 µm and the longest cell can reach from the toe to the lower brain stem (Pseudounipolar cells). The largest known cells are unfertilised ostrich egg cells which weigh 3.3 pounds.

In 1835, before the final cell theory was developed, Jan Evangelista Purkyně observed small "granules" while looking at the plant tissue through a microscope. The cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that all cells come from preexisting cells, that vital functions of an organism occur within cells, and that all cells contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells.

The word *cell* comes from the Latin *cellula*, meaning, a small room. The descriptive term for the smallest living biological structure was coined by Robert Hooke in a book he published in 1665 when he compared the cork cells he saw through his microscope to the small rooms monks lived in.

Anatomy

There are two types of cells: eukaryotic and prokaryotic. Prokaryotic cells are usually independent, while eukaryotic cells are often found in multicellular organisms.

Prokaryotic cells

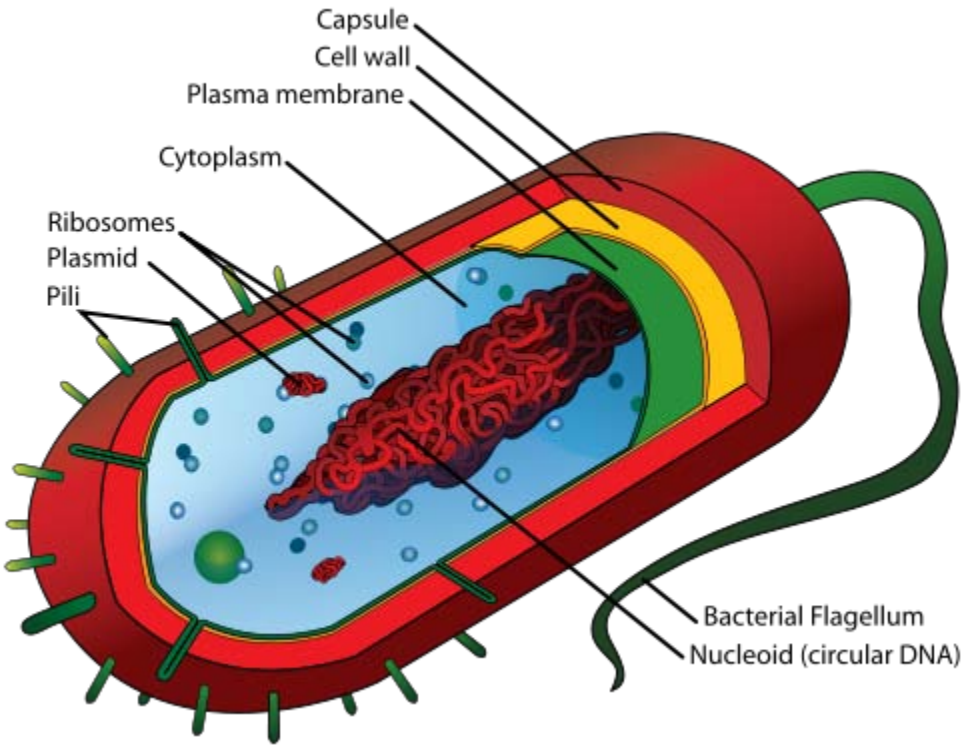


Diagram of a typical prokaryotic cell

The prokaryote cell is simpler, and therefore smaller, than a eukaryote cell, lacking a nucleus and most of the other organelles of eukaryotes. There are two kinds of prokaryotes: bacteria and archaea; these share a similar structure.

Nuclear material of prokaryotic cell consist of a single chromosome which is in direct contact with cytoplasm. Here the undefined nuclear region in the cytoplasm is called nucleoid.

A prokaryotic cell has three architectural regions:

- On the outside, flagella and pili project from the cell's surface. These are structures (not present in all prokaryotes) made of proteins that facilitate movement and communication between cells;
- Enclosing the cell is the cell envelope – generally consisting of a cell wall covering a plasma membrane though some bacteria also have a further covering layer called a capsule. The envelope gives rigidity to the cell and separates the interior of the cell from its environment, serving as a protective filter. Though most prokaryotes have a cell wall, there are exceptions such as *Mycoplasma* (bacteria) and *Thermoplasma* (archaea). The cell wall consists of *peptidoglycan* in bacteria, and acts as an additional barrier against exterior forces. It also prevents

the cell from expanding and finally bursting (cytolysis) from osmotic pressure against a hypotonic environment. Some eukaryote cells (plant cells and fungi cells) also have a cell wall;

- Inside the cell is the cytoplasmic region that contains the cell genome (DNA) and ribosomes and various sorts of inclusions. A prokaryotic chromosome is usually a circular molecule (an exception is that of the bacterium *Borrelia burgdorferi*, which causes Lyme disease). Though not forming a *nucleus*, the DNA is condensed in a *nucleoid*. Prokaryotes can carry extrachromosomal DNA elements called *plasmids*, which are usually circular. Plasmids enable additional functions, such as antibiotic resistance.

Eukaryotic cells

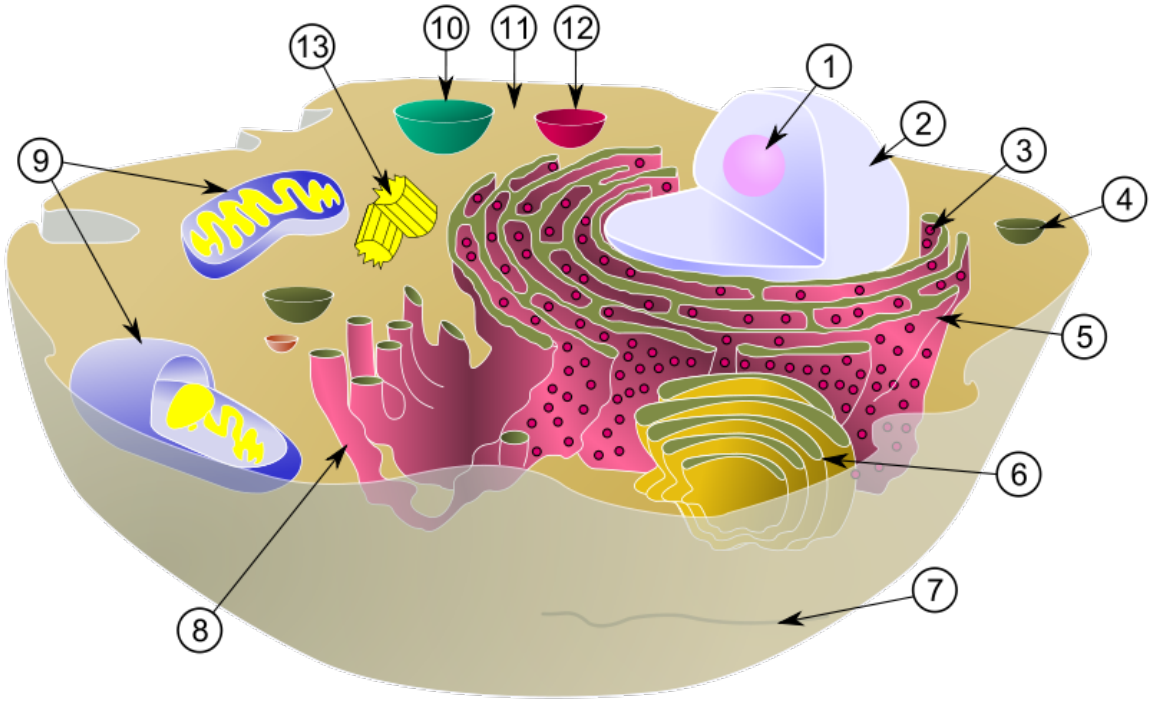


Diagram of a typical animal (eukaryotic) cell, showing subcellular components.
Organelles:
(1) nucleolus
(2) nucleus
(3) ribosome
(4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) smooth endoplasmic reticulum
(9) mitochondria
(10) vacuole
(11) cytoplasm

(12) lysosome
(13) centrioles within centrosome

Eukaryotic cells are about 15 times wider than a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. This nucleus gives the eukaryote its name, which means "true nucleus." Other differences include:

- The plasma membrane resembles that of prokaryotes in function, with minor differences in the setup. Cell walls may or may not be present.
- The eukaryotic DNA is organized in one or more linear molecules, called chromosomes, which are associated with histone proteins. All chromosomal DNA is stored in the *cell nucleus*, separated from the cytoplasm by a membrane. Some eukaryotic organelles such as mitochondria also contain some DNA.
- Many eukaryotic cells are ciliated with *primary cilia*. Primary cilia play important roles in chemosensation, mechanosensation, and thermosensation. Cilia may thus be "viewed as sensory cellular antennae that coordinate a large number of cellular signaling pathways, sometimes coupling the signaling to ciliary motility or alternatively to cell division and differentiation."
- Eukaryotes can move using *motile cilia* or *flagella*. The flagella are more complex than those of prokaryotes.

Table 1: Comparison of features of prokaryotic and eukaryotic cells

	Prokaryotes	Eukaryotes
Typical organisms	bacteria, archaea	protists, fungi, plants, animals
Typical size	~ 1–10 μm	~ 10–100 μm (sperm cells, apart from the tail, are smaller)
Type of nucleus	nucleoid region; no real nucleus	real nucleus with double membrane
DNA	circular (usually)	linear molecules (chromosomes) with histone proteins
RNA-/protein-synthesis	coupled in cytoplasm	RNA-synthesis inside the nucleus protein synthesis in cytoplasm
Ribosomes	50S+30S	60S+40S
Cytoplasmatic structure	very few structures	highly structured by endomembranes and a cytoskeleton
Cell movement	flagella made of flagellin	flagella and cilia containing microtubules; lamellipodia and filopodia containing actin
Mitochondria	none	one to several thousand (though some lack mitochondria)
Chloroplasts	none	in algae and plants
Organization	usually single cells	single cells, colonies, higher multicellular

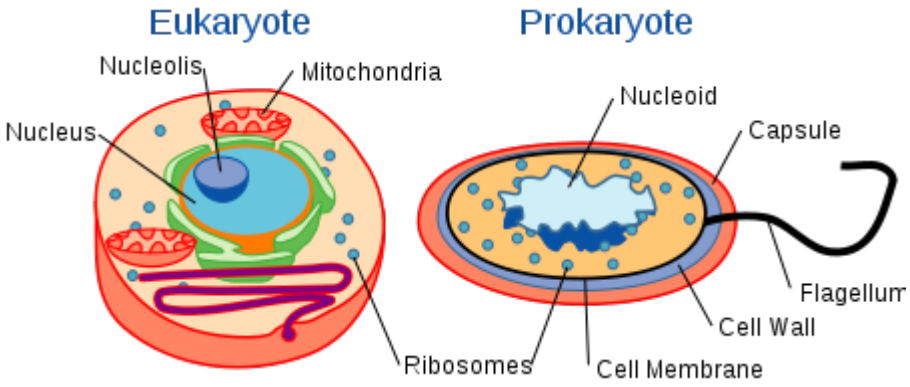
		organisms with specialized cells
Cell division	Binary fission (simple division)	Mitosis (fission or budding) Meiosis

Table 2: Comparison of structures between animal and plant cells

	Typical animal cell	Typical plant cell

Organelles	• Nucleus
	  ○ Nucleolus (within nucleus)
	• Rough endoplasmic reticulum (ER)
	• Smooth ER
	• Ribosomes
	• Cytoskeleton
	• Golgi apparatus
	• Cytoplasm
	• Mitochondria
	• Vesicles
	• Lysosomes
	• Centrosome
	  ○ Centrioles
		• Nucleus
		  ○ Nucleolus (within nucleus)
		• Rough ER
		• Smooth ER
		• Ribosomes
		• Cytoskeleton
		• Golgi apparatus (dictiosomes)
		• Cytoplasm
		• Mitochondria
		• Plastids and its derivatives
		• Vacuole(s)
		• Cell wall

Subcellular components



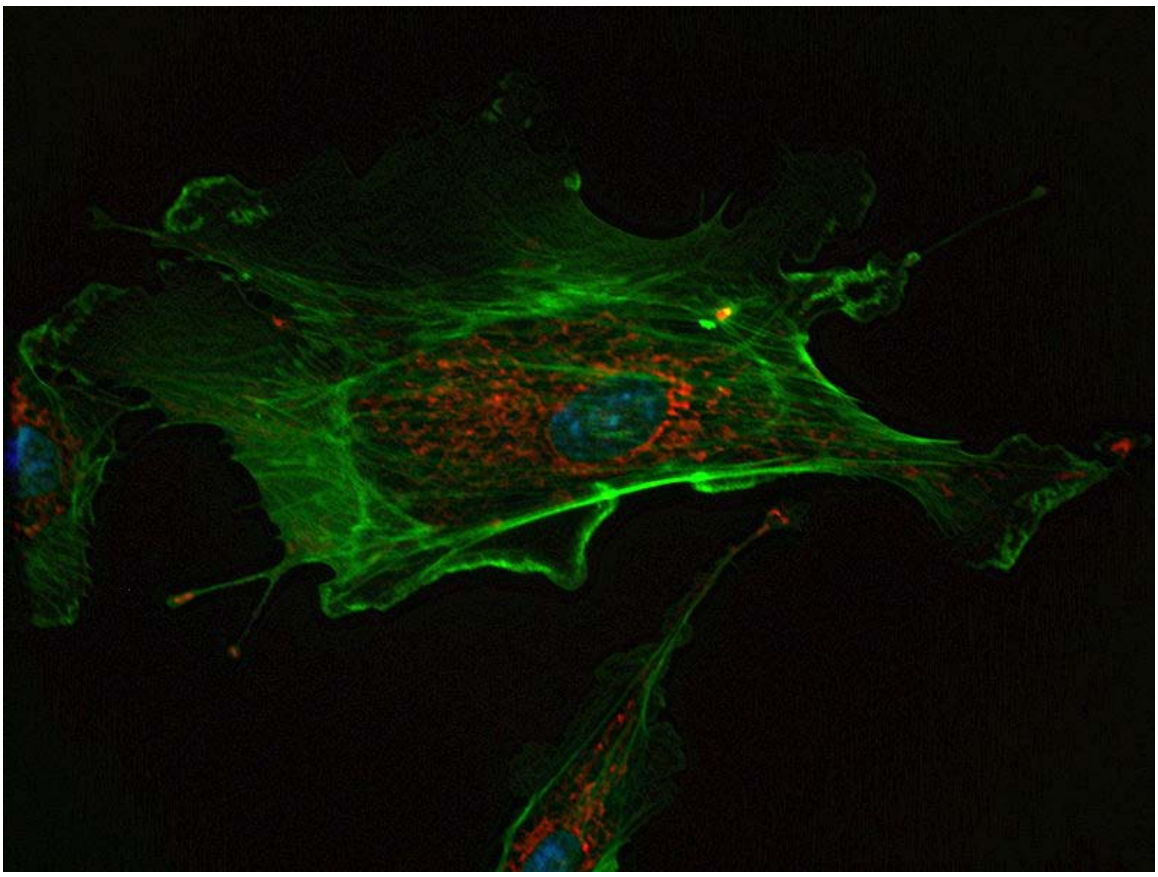
The cells of eukaryotes (left) and prokaryotes (right)

All cells, whether prokaryotic or eukaryotic, have a membrane that envelops the cell, separates its interior from its environment, regulates what moves in and out (selectively permeable), and maintains the electric potential of the cell. Inside the membrane, a salty cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells.

Membrane

The cytoplasm of a cell is surrounded by a cell membrane or *plasma membrane*. The plasma membrane in plants and prokaryotes is usually covered by a cell wall. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of lipids (hydrophobic fat-like molecules) and hydrophilic phosphorus molecules. Hence, the layer is called a phospholipid bilayer. It may also be called a fluid mosaic membrane. Embedded within this membrane is a variety of protein molecules that act as channels and pumps that move different molecules into and out of the cell. The membrane is said to be 'semi-permeable', in that it can either let a substance (molecule or ion) pass through freely, pass through to a limited extent or not pass through at all. Cell surface membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones.

Cytoskeleton



Bovine Pulmonary Artery Endothelial cell: nuclei stained blue, mitochondria stained red, and F-actin, an important component in microfilaments, stained green. Cell imaged on a fluorescent microscope.

The cytoskeleton acts to organize and maintain the cell's shape; anchors organelles in place; helps during endocytosis, the uptake of external materials by a cell, and cytokinesis, the separation of daughter cells after cell division; and moves parts of the

cell in processes of growth and mobility. The eukaryotic cytoskeleton is composed of microfilaments, intermediate filaments and microtubules. There is a great number of proteins associated with them, each controlling a cell's structure by directing, bundling, and aligning filaments. The prokaryotic cytoskeleton is less well-studied but is involved in the maintenance of cell shape, polarity and cytokinesis.

Genetic material

Two different kinds of genetic material exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most organisms use DNA for their long-term information storage, but some viruses (e.g., retroviruses) have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence. RNA is also used for information transport (e.g., mRNA) and enzymatic functions (e.g., ribosomal RNA) in organisms that use DNA for the genetic code itself. Transfer RNA (tRNA) molecules are used to add amino acids during protein translation.

Prokaryotic genetic material is organized in a simple circular DNA molecule (the bacterial chromosome) in the nucleoid region of the cytoplasm. Eukaryotic genetic material is divided into different, linear molecules called chromosomes inside a discrete nucleus, usually with additional genetic material in some organelles like mitochondria and chloroplasts.

A human cell has genetic material contained in the cell nucleus (the nuclear genome) and in the mitochondria (the mitochondrial genome). In humans the nuclear genome is divided into 23 pairs of linear DNA molecules called chromosomes. The mitochondrial genome is a circular DNA molecule distinct from the nuclear DNA. Although the mitochondrial DNA is very small compared to nuclear chromosomes, it codes for 13 proteins involved in mitochondrial energy production and specific tRNAs.

Foreign genetic material (most commonly DNA) can also be artificially introduced into the cell by a process called transfection. This can be transient, if the DNA is not inserted into the cell's genome, or stable, if it is. Certain viruses also insert their genetic material into the genome.

Organelles

The human body contains many different organs, such as the heart, lung, and kidney, with each organ performing a different function. Cells also have a set of "little organs," called organelles, that are adapted and/or specialized for carrying out one or more vital functions. Both eukaryotic and prokaryotic cells have organelles but organelles in eukaryotes are generally more complex and may be membrane bound.

There are several types of organelles in a cell. Some (such as the nucleus and golgi apparatus) are typically solitary, while others (such as mitochondria, peroxisomes and lysosomes) can be numerous (hundreds to thousands). The cytosol is the gelatinous fluid that fills the cell and surrounds the organelles.

Cell nucleus – eukaryotes only - a cell's information center

The cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis (transcription) occur. The nucleus is spherical and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called messenger RNA (mRNA). This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule. The nucleolus is a specialized region within the nucleus where ribosome subunits are assembled. In prokaryotes, DNA processing takes place in the cytoplasm.

Diagram of a cell nucleus

Mitochondria and Chloroplasts – eukaryotes only - the power generators

Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. Mitochondria play a critical role in generating energy in the eukaryotic cell. Mitochondria generate the cell's energy by oxidative phosphorylation, using oxygen to release energy stored in cellular nutrients (typically pertaining to glucose) to generate ATP. Mitochondria multiply by splitting in two. Respiration occurs in the cell mitochondria. Organelles that are modified chloroplasts are broadly called plastids, and are involved in energy storage through photosynthesis, which uses solar energy to generate carbohydrates and oxygen from carbon dioxide and water. Mitochondria and chloroplasts each contain their own genome, which is separate and distinct from the nuclear genome of a cell. Both organelles contain this DNA in circular plasmids, much like prokaryotic cells, strongly supporting the evolutionary theory of endosymbiosis; since these organelles contain

their own genomes and have other similarities to prokaryotes, they are thought to have developed through a symbiotic relationship after being engulfed by a primitive cell.

Endoplasmic reticulum – eukaryotes only

The endoplasmic reticulum (ER) is the transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that will float freely in the cytoplasm. The ER has two forms: the rough ER, which has ribosomes on its surface and secretes proteins into the cytoplasm, and the smooth ER, which lacks them. Smooth ER plays a role in calcium sequestration and release.

Golgi apparatus – eukaryotes only

The primary function of the Golgi apparatus is to process and package the macromolecules such as proteins and lipids that are synthesized by the cell. It is particularly important in the processing of proteins for secretion. The Golgi apparatus forms a part of the endomembrane system of eukaryotic cells. Vesicles that enter the Golgi apparatus are processed in a cis to trans direction, meaning they coalesce on the cis side of the apparatus and after processing pinch off on the opposite (trans) side to form a new vesicle in the animal cell.

Diagram of an endomembrane system

Ribosomes

The ribosome is a large complex of RNA and protein molecules. They each consist of two subunits, and act as an assembly line where RNA from the nucleus is used to synthesise proteins from amino acids. Ribosomes can be found either floating freely or bound to a membrane (the rough endoplasmatic reticulum in eukaryotes, or the cell membrane in prokaryotes).

Lysosomes and Peroxisomes – eukaryotes only

Lysosomes contain digestive enzymes (acid hydrolases). They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. Peroxisomes have enzymes that rid the cell of toxic peroxides. The cell could not house these

destructive enzymes if they were not contained in a membrane-bound system. These organelles are often called a "suicide bag" because of their ability to detonate and destroy the cell.

Centrosome – the cytoskeleton organiser

The centrosome produces the microtubules of a cell – a key component of the cytoskeleton. It directs the transport through the ER and the Golgi apparatus. Centrosomes are composed of two centrioles, which separate during cell division and help in the formation of the mitotic spindle. A single centrosome is present in the animal cells. They are also found in some fungi and algae cells.

Vacuoles

Vacuoles store food and waste. Some vacuoles store extra water. They are often described as liquid filled space and are surrounded by a membrane. Some cells, most notably *Amoeba*, have contractile vacuoles, which can pump water out of the cell if there is too much water. The vacuoles of eukaryotic cells are usually larger in those of plants than animals.

Structures outside the cell wall

Capsule

A gelatinous capsule is present in some bacteria outside the cell wall. The capsule may be polysaccharide as in pneumococci, meningococci or polypeptide as *Bacillus anthracis* or hyaluronic acid as in streptococci. Capsules are not marked by ordinary stain and can be detected by special stain. The capsule is antigenic. The capsule has antiphagocytic function so it determines the virulence of many bacteria. It also plays a role in attachment of the organism to mucous membranes.

Flagella

Flagella are the organelles of cellular mobility. They arise from cytoplasm and extrude through the cell wall. They are long and thick thread-like appendages, protein in nature. Are most commonly found in bacteria cells but are found in animal cells as well.

Fimbriae (pili)

They are short and thin hair like filaments, formed of protein called pilin (antigenic). Fimbriae are responsible for attachment of bacteria to specific receptors of human cell (adherence). There are special types of pili called (sex pili) involved in conjunction.

Functions

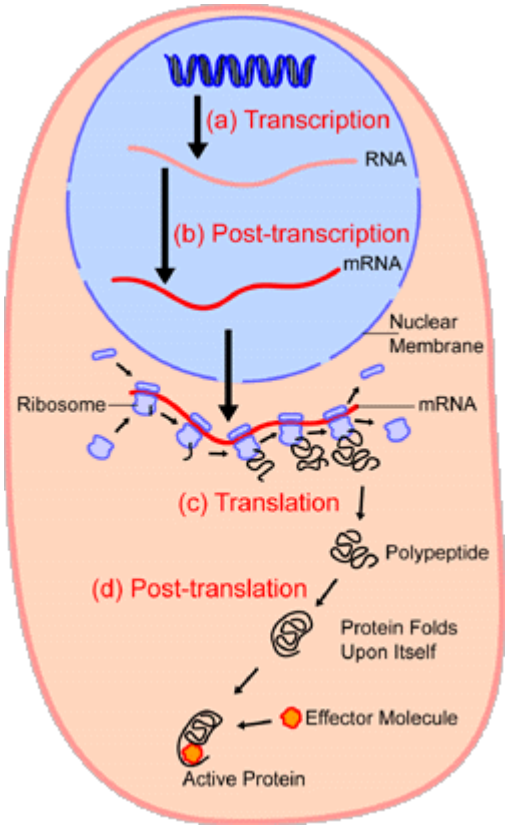
Growth and metabolism

Between successive cell divisions, cells grow through the functioning of cellular metabolism. Cell metabolism is the process by which individual cells process nutrient

molecules. Metabolism has two distinct divisions: catabolism, in which the cell breaks down complex molecules to produce energy and reducing power, and anabolism, in which the cell uses energy and reducing power to construct complex molecules and perform other biological functions. Complex sugars consumed by the organism can be broken down into a less chemically complex sugar molecule called glucose. Once inside the cell, glucose is broken down to make adenosine triphosphate (ATP), a form of energy, through two different pathways.

The first pathway, glycolysis, requires no oxygen and is referred to as anaerobic metabolism. Each reaction is designed to produce some hydrogen ions that can then be used to make energy packets (ATP). In prokaryotes, glycolysis is the only method used for converting energy.

The second pathway, called the Krebs cycle, or citric acid cycle, occurs inside the mitochondria and can generate enough ATP to run all the cell functions.



An overview of protein synthesis.
Within the nucleus of the cell (*light blue*), genes (DNA, *dark blue*) are transcribed into RNA. This RNA is then subject to post-transcriptional modification and control, resulting in a mature mRNA (*red*) that is then transported out of the nucleus and into the cytoplasm (*peach*), where it undergoes translation into a protein. mRNA is translated by ribosomes (*purple*) that match the three-base codons of the mRNA to the three-base anti-codons of the appropriate tRNA. Newly synthesized proteins (*black*) are often further modified, such as by binding to an effector molecule (*orange*), to become fully active.

Creation

Cell division involves a single cell (called a *mother cell*) dividing into two daughter cells. This leads to growth in multicellular organisms (the growth of tissue) and to procreation (vegetative reproduction) in unicellular organisms.

Prokaryotic cells divide by binary fission. Eukaryotic cells usually undergo a process of nuclear division, called mitosis, followed by division of the cell, called cytokinesis. A diploid cell may also undergo meiosis to produce haploid cells, usually four. Haploid cells serve as gametes in multicellular organisms, fusing to form new diploid cells.

DNA replication, or the process of duplicating a cell's genome, is required every time a cell divides. Replication, like all cellular activities, requires specialized proteins for carrying out the job.

Protein synthesis

Cells are capable of synthesizing new proteins, which are essential for the modulation and maintenance of cellular activities. This process involves the formation of new protein molecules from amino acid building blocks based on information encoded in DNA/RNA. Protein synthesis generally consists of two major steps: transcription and translation.

Transcription is the process where genetic information in DNA is used to produce a complementary RNA strand. This RNA strand is then processed to give messenger RNA (mRNA), which is free to migrate through the cell. mRNA molecules bind to protein-RNA complexes called ribosomes located in the cytosol, where they are translated into polypeptide sequences. The ribosome mediates the formation of a polypeptide sequence based on the mRNA sequence. The mRNA sequence directly relates to the polypeptide sequence by binding to transfer RNA (tRNA) adapter molecules in binding pockets within the ribosome. The new polypeptide then folds into a functional three-dimensional protein molecule.

Movement or motility

Cells can move during many processes: such as wound healing, the immune response and cancer metastasis. For wound healing to occur, white blood cells and cells that ingest bacteria move to the wound site to kill the microorganisms that cause infection.
At the same time fibroblasts (connective tissue cells) move there to remodel damaged structures. In the case of tumor development, cells from a primary tumor move away and spread to other parts of the body. Cell motility involves many receptors, crosslinking, bundling, binding, adhesion, motor and other proteins. The process is divided into three steps – protrusion of the leading edge of the cell, adhesion of the leading edge and de-adhesion at the cell body and rear, and cytoskeletal contraction to pull the cell forward. Each step is driven by physical forces generated by unique segments of the cytoskeleton.

Evolution

The origin of cells has to do with the origin of life, which began the history of life on Earth.

Origin of the first cell

There are several theories about the origin of small molecules that could lead to life in an early Earth. One is that they came from meteorites. Another is that they were created at deep-sea vents. A third is that they were synthesized by lightning in a reducing atmosphere; although it is not clear if Earth had such an atmosphere. There are essentially no experimental data defining what the first self-replicating forms were. RNA is generally assumed to be the earliest self-replicating molecule, as it is capable of both storing genetic information and catalyzing chemical reactions. But some other entity with the potential to self-replicate could have preceded RNA, like clay or peptide nucleic acid.

Cells emerged at least 4.0–4.3 billion years ago. The current belief is that these cells were heterotrophs. An important characteristic of cells is the cell membrane, composed of a bilayer of lipids. The early cell membranes were probably more simple and permeable than modern ones, with only a single fatty acid chain per lipid. Lipids are known to spontaneously form bilayered vesicles in water, and could have preceded RNA. But the first cell membranes could also have been produced by catalytic RNA, or even have required structural proteins before they could form.

Origin of eukaryotic cells

The eukaryotic cell seems to have evolved from a symbiotic community of prokaryotic cells. DNA-bearing organelles like the mitochondria and the chloroplasts are almost certainly what remains of ancient symbiotic oxygen-breathing proteobacteria and cyanobacteria, respectively, where the rest of the cell seems to be derived from an ancestral archaean prokaryote cell – a theory termed the endosymbiotic theory.

There is still considerable debate about whether organelles like the hydrogenosome predated the origin of mitochondria, or viceversa.

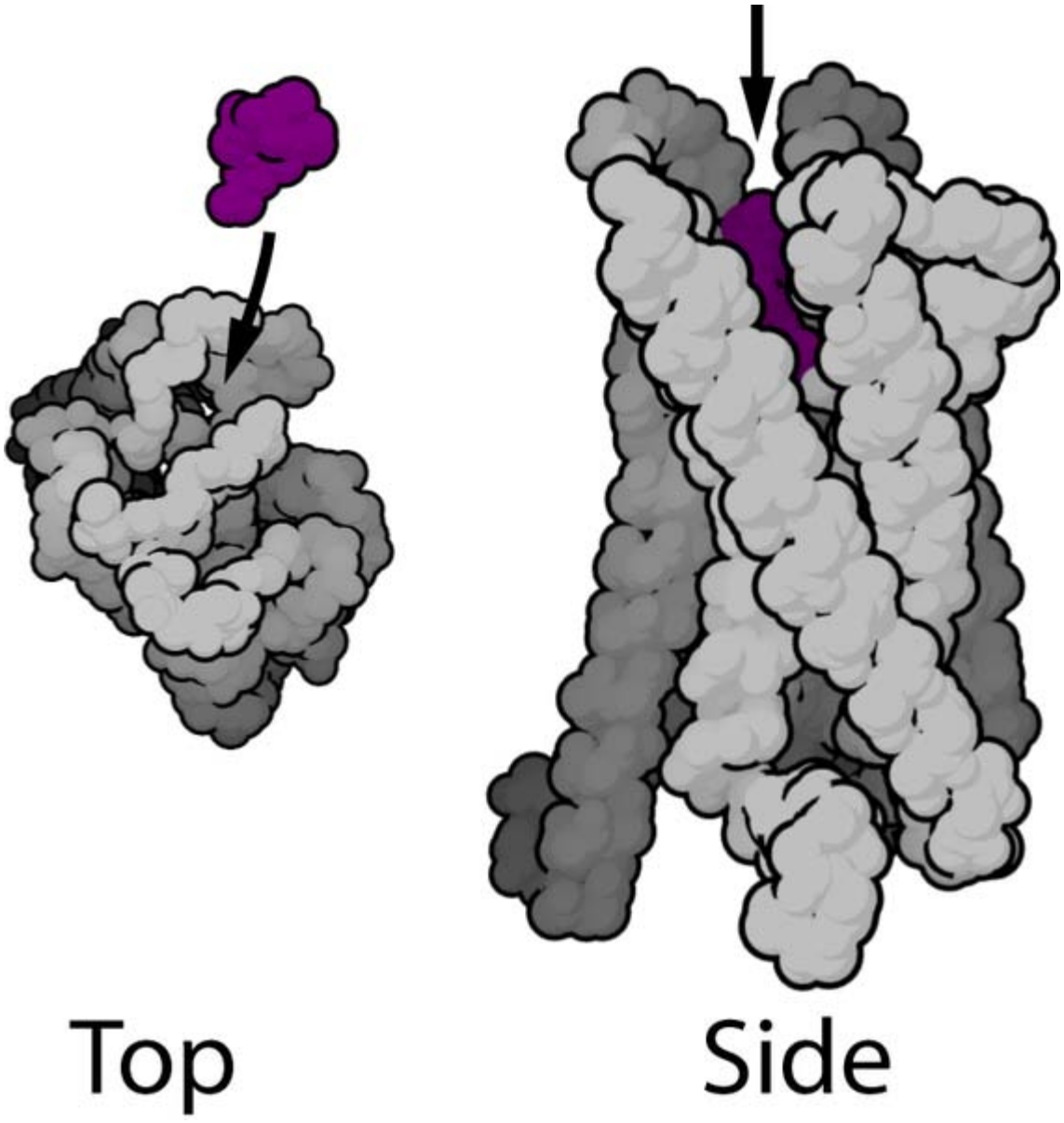
Sex, as the stereotyped choreography of meiosis and syngamy that persists in nearly all extant eukaryotes, may have played a role in the transition from prokaryotes to eukaryotes. An 'origin of sex as vaccination' theory suggests that the eukaryote genome accreted from prokaryan parasite genomes in numerous rounds of lateral gene transfer. Sex-as-syngamy (fusion sex) arose when infected hosts began swapping nuclearized genomes containing co-evolved, vertically transmitted symbionts that conveyed protection against horizontal infection by more virulent symbionts.

History

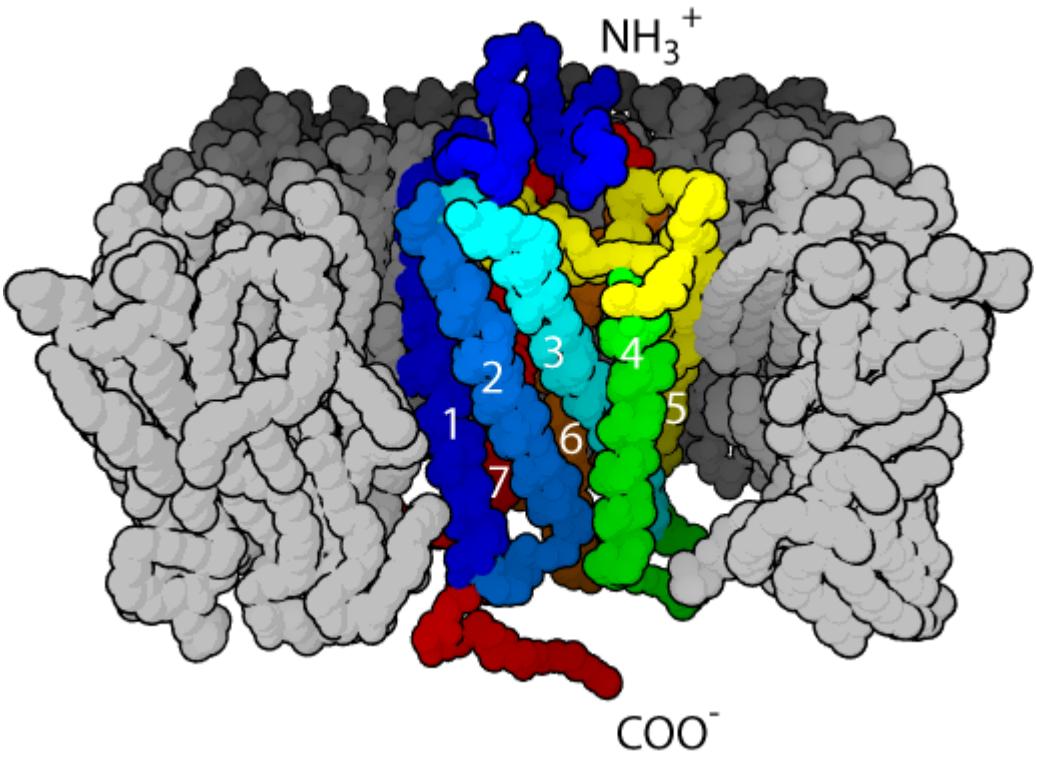
- 1632–1723: Antonie van Leeuwenhoek teaches himself to grind lenses, builds a microscope and draws protozoa, such as *Vorticella* from rain water, and bacteria from his own mouth.
- 1665: Robert Hooke discovers cells in cork, then in living plant tissue using an early microscope.
- 1839: Theodor Schwann and Matthias Jakob Schleiden elucidate the principle that plants and animals are made of cells, concluding that cells are a common unit of structure and development, and thus founding the cell theory.
- The belief that life forms can occur spontaneously (*generatio spontanea*) is contradicted by Louis Pasteur (1822–1895) (although Francesco Redi had performed an experiment in 1668 that suggested the same conclusion).
- 1855: Rudolf Virchow states that cells always emerge from cell divisions (*omnis cellula ex cellula*).
- 1931: Ernst Ruska builds first transmission electron microscope (TEM) at the University of Berlin. By 1935, he has built an EM with twice the resolution of a light microscope, revealing previously unresolvable organelles.
- 1953: Watson and Crick made their first announcement on the double-helix structure for DNA on February 28.
- 1981: Lynn Margulis published *Symbiosis in Cell Evolution* detailing the endosymbiotic theory.

Chapter- 3

G protein-Coupled Receptor



A mu-opioid G-protein-coupled receptor with its agonist

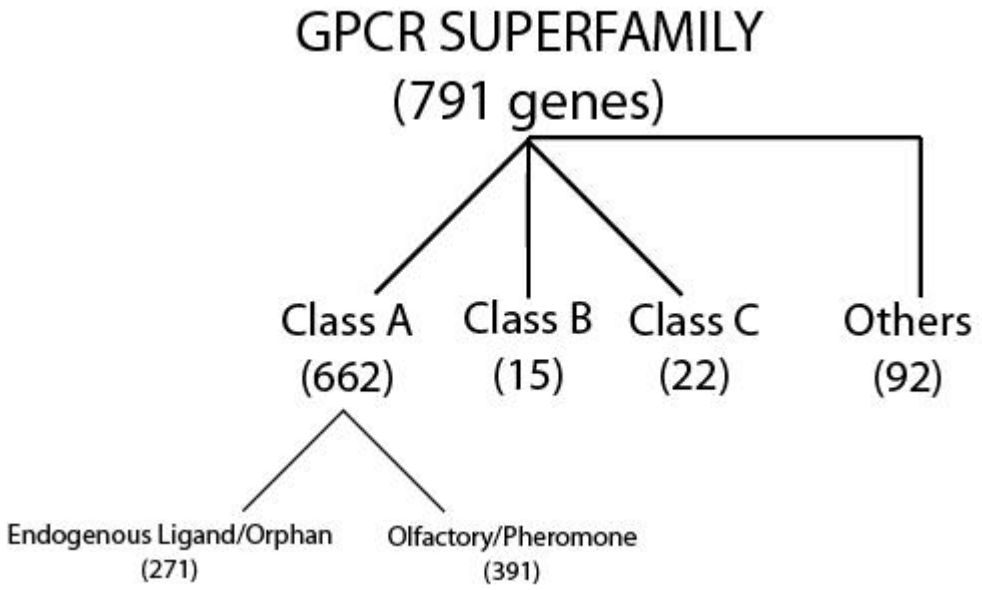


The seven-transmembrane α-helix structure of a G-protein-coupled receptor

G protein-coupled receptors (**GPCRs**), also known as **seven-transmembrane domain receptors**, **7TM receptors**, **heptahelical receptors**, **serpentine receptor**, and **G protein-linked receptors** (**GPLR**), comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses. G protein-coupled receptors are found only in eukaryotes, including yeast, choanoflagellates, and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases, and are also the target of approximately 30% of all modern medicinal drugs.

There are two principal signal transduction pathways involving the G protein-coupled receptors: the cAMP signal pathway and the Phosphatidylinositol signal pathway. When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR can then activate an associated G-protein by exchanging its bound GDP for a GTP. The G-protein's α subunit, together with the bound GTP, can then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$).

Classification



Classification Scheme of GPCRs. Class A (Rhodopsin-like), Class B (Secretin-like),Class C (Glutamate Receptor-like), Others (Adhesion (33), Frizzled (11), Taste type-2 (25), unclassified (23)).

The exact size of the GPCR superfamily is unknown but nearly 800 different human genes (or ≈4% of the entire protein-coding genome) have been predicted from genome sequence analysis. Although numerous classification schemes have been proposed, the superfamily is classically divided into three main classes (A, B, and C) with no detectable shared sequence homology between classes. The largest class by far is class A, which accounts for nearly 85% of the GPCR genes. Of class A GPCRs, over half of these are predicted to encode olfactory receptors while the remaining receptors are liganded by known endogenous compounds or are classified as orphan receptors. Despite the lack of sequence homology between classes, all GPCRs share a common structure and mechanism of signal transduction.

In all, GPCRs can be grouped into 6 classes based on sequence homology and functional similarity:

- Class A (or 1) (Rhodopsin-like)
- Class B (or 2) (Secretin receptor family)
- Class C (or 3) (Metabotropic glutamate/pheromone)
- Class D (or 4) (Fungal mating pheromone receptors)
- Class E (or 5) (Cyclic AMP receptors)
- Class F (or 6) (Frizzled/Smoothened)

The very large rhodopsin A group has been further subdivided into 19 subgroups (A1-A19). More recently, an alternative classification system called GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, Secretin) has been proposed.

The human genome encodes thousands of G protein-coupled receptors, about 350 of which detect hormones, growth factors, and other endogenous ligands. Approximately 150 of the GPCRs found in the human genome have unknown functions.

Some web-servers and bioinformatics prediction methods have been used for predicting the classification of GPCRs according to their amino acid sequence alone, by means of the pseudo amino acid composition approach.

Physiological roles

GPCRs are involved in a wide variety of physiological processes. Some examples of their physiological roles include:

1. the visual sense: the opsins use a photoisomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of *11-cis*-retinal to *all-trans*-retinal for this purpose
2. the sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
3. behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, GABA, and glutamate
4. regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response
5. autonomic nervous system transmission: both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes
6. cell density sensing: A novel GPCR role in regulating cell density sensing.

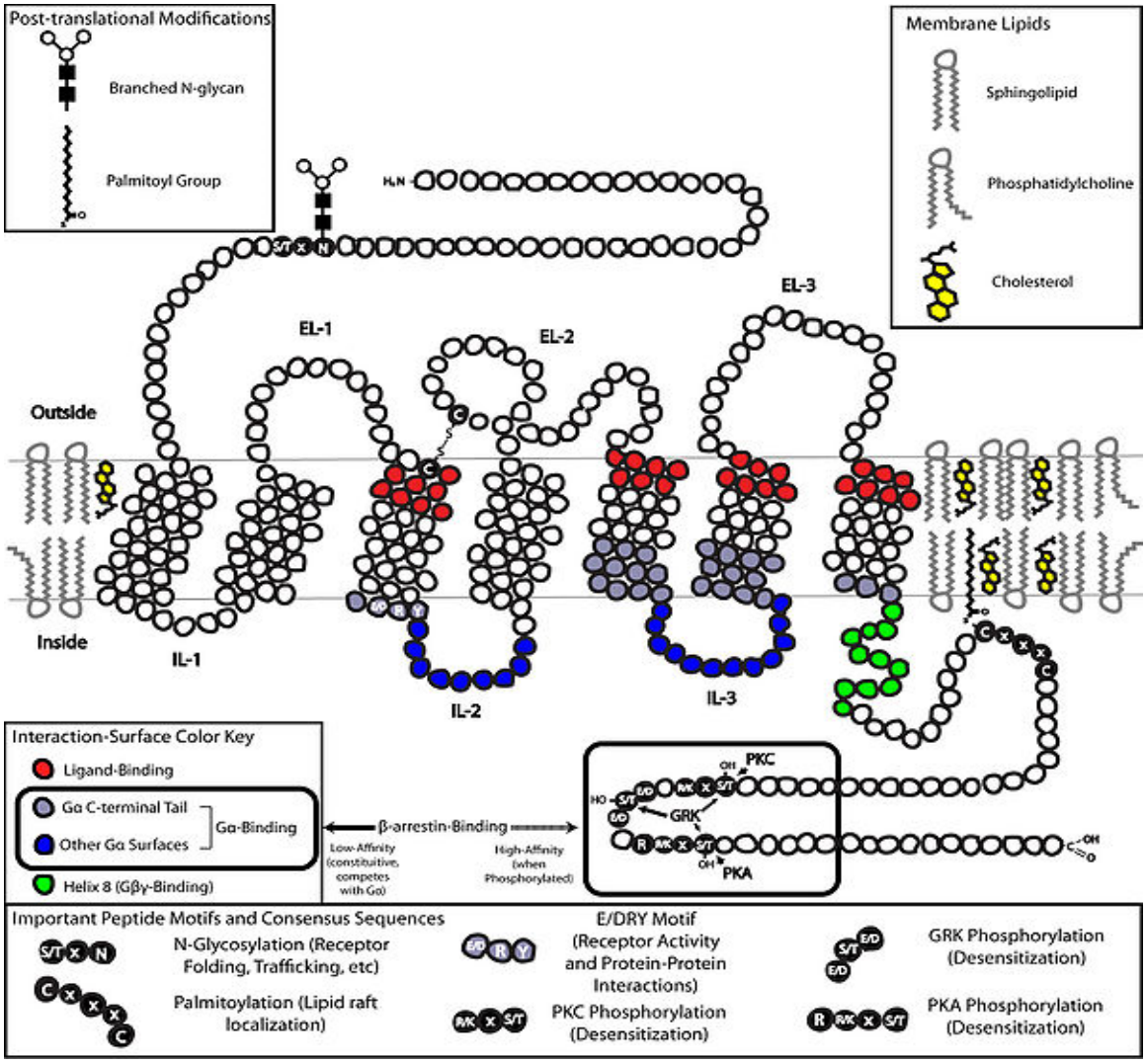
Receptor structure

GPCRs are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly-conserved cysteine residues that form disulfide bonds to stabilize the receptor structure. Some seven-transmembrane helix proteins (channelrhodopsin) that resemble GPCRs may contain ion channels, within their protein.

Early structural models for GPCRs were based on their weak analogy to bacteriorhodopsin, for which a structure had been determined by both electron diffraction (PDB 2BRD, 1AT9) and X ray-based crystallography (1AP9). In 2000, the first crystal

structure of a mammalian GPCR, that of bovine rhodopsin (1F88), was solved. While the main feature, the seven transmembrane helices, is conserved, the relative orientation of the helices differ significantly from that of bacteriorhodopsin. In 2007, the first structure of a human GPCR was solved (2R4R, 2R4S). This was followed immediately by a higher resolution structure of the same receptor (2RH1). This human β_2-adrenergic receptor GPCR structure, proved to be highly similar to the bovine rhodopsin in terms of the relative orientation of the seven-transmembrane helices. However the conformation of the second extracellular loop is entirely different between the two structures. Since this loop constitutes the "lid" that covers the top of the ligand binding site, this conformational difference highlights the difficulties in constructing homology models of other GPCRs based only on the rhodopsin structure.

Structure-function relationships



Two-dimensional schematic of a generic GPCR set in a Lipid Raft.

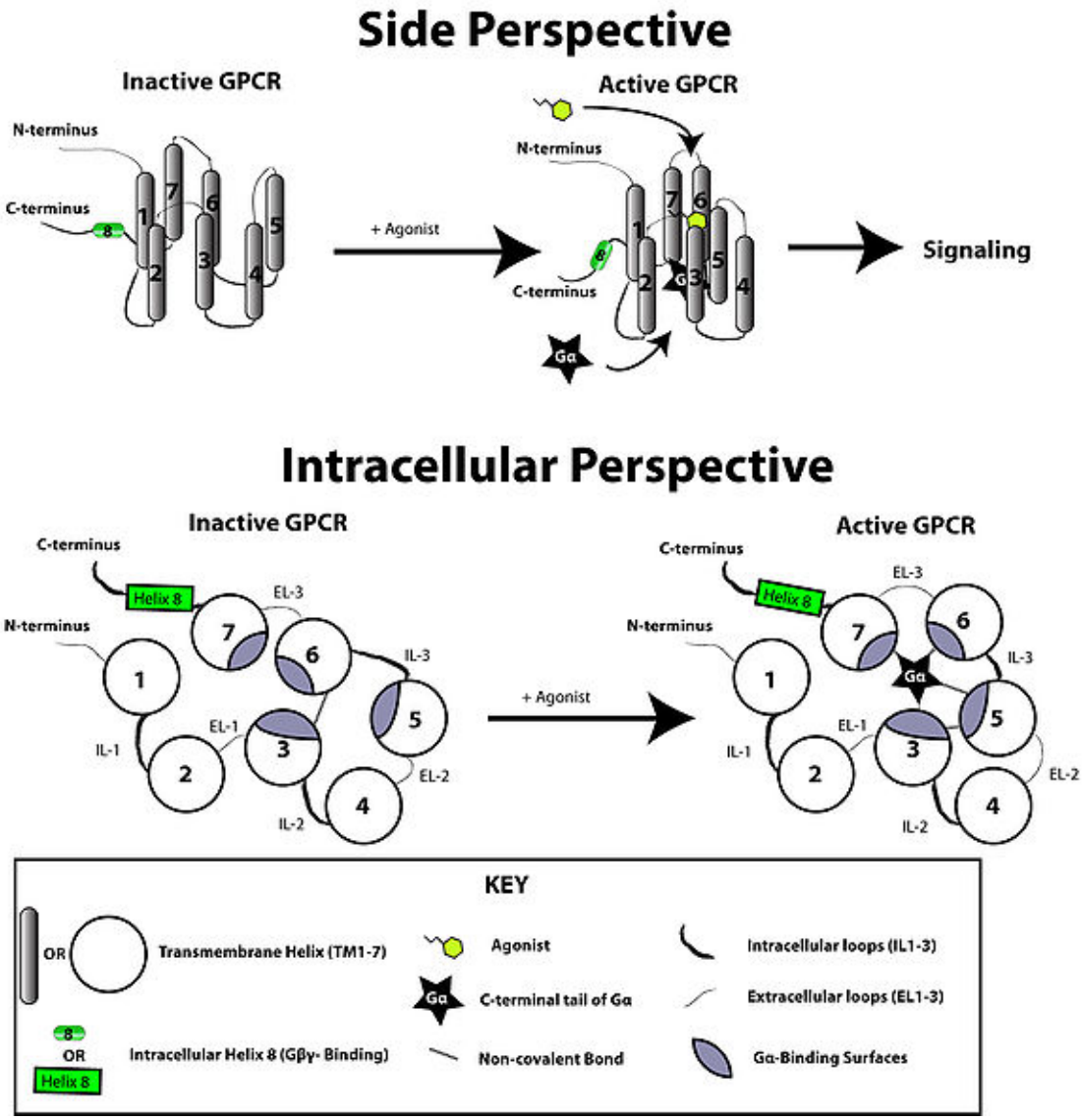
Structurally GPCRs are characterized by an extracellular N-terminus, followed by seven transmembrane (7-TM) α-helices (TM-1 to TM-7) connected by three intracellular (IL-1 to IL-3) and three extracellular loops (EL-1 to EL-3), and finally an intracellular C-terminus. The GPCR arranges itself into a tertiary structure resembling a barrel, with the seven transmembrane helices forming a cavity within the plasma membrane which serves a ligand-binding domain that is often covered by EL-2. Ligands may also bind elsewhere, however, as is the case for bulkier ligands (e.g., proteins or large peptides) which instead interact with the extracellular loops, or, as illustrated by the class C metabotropic glutamate receptors (mGluRs), the N-terminal tail. The class C GPCRs are distinguished by their large N-terminal tail, which also contains a ligand-binding domain. Upon glutamate-binding to an mGluR, the N-terminal tail undergoes a conformational change that leads to its interaction with the residues of the extracellular loops and TM domains. The eventual effect of all three types of agonist-induced activation is a change in the relative orientations of the TM helices (likened to a twisting motion) leading to a wider intracellular surface and "revelation" of residues of the intracellular helices and TM domains crucial to signal transduction function (i.e., G-protein coupling). Inverse agonists and antagonists may also bind to a number of different sites, but the eventual effect must be prevention of this TM helix reorientation.

The structure of the N- and C-terminal tails of GPCRs may also serve important functions beyond ligand-binding. In particular, the C-terminus often contains serine (Ser) or threonine (Thr) residues that, when phosphorylated, increase the affinity of the intracellular surface for the binding of scaffolding proteins called β-arrestins (β-arr). Once bound, β-arrestins both sterically prevent G-protein coupling and may recruit other proteins leading to the creation of signaling complexes involved in extracellular-signal regulated kinase (ERK) pathway activation or receptor endocytosis (internalization). As the phosphorylation of these Ser and Thr residues often occurs as a result of GPCR activation, the β-arr-mediated G-protein-decoupling and internalization of GPCRs are important mechanisms of desensitization.

A final common structural theme amongst GPCRs is palmitoylation of one or more sites of the C-terminal tail or the intracellular loops. Palmitoylation is the covalent modification of cysteine (Cys) residues via addition of hydrophobic acyl groups, and has the effect of targeting the receptor to cholesterol- and sphingolipid-rich microdomains of the plasma membrane called lipid rafts. As many of the downstream transducer and effector molecules of GPCRs (including those involved in negative feedback pathways) are also targeted to lipid rafts, this has the effect of facilitating rapid receptor signaling.

GPCRs respond to extracellular signals mediated by a huge diversity of agonists, ranging from proteins to biogenic amines to protons, but all transduce this signal via a mechanism of G-protein coupling. This is made possible by virtue of a guanine-nucleotide exchange factor (GEF) domain primarily formed by a combination of IL-2 and IL-3 along with adjacent residues of the associated TM helices.

Mechanism



Cartoon depicting the basic concept of GPCR Conformational Activation. Ligand binding disrupts an ionic lock between the E/DRY motif of TM-3 and acidic residues of TM-6. As a result the GPCR reorganizes to allow activation of G-alpha proteins. The side perspective is a view from above and to the side of the GPCR as it is set in the plasma membrane (the membrane lipids have been omitted for clarity). The intracellular perspective shows the view looking up at the plasma membrane from inside the cell.

The G protein-coupled receptor is activated by an external signal in the form of a ligand or other signal mediator. This creates a conformational change in the receptor, causing activation of a G protein. Further effect depends on the type of G protein.

Ligand binding

GPCRs include receptors for sensory signal mediators (e.g., light and olfactory stimulatory molecules); adenosine, bombesin, bradykinin, endothelin, γ-aminobutyric acid (GABA), hepatocyte growth factor (HGF), melanocortins, neuropeptide Y, opioid peptides, opsins, somatostatin, tachykinins, members of the vasoactive intestinal peptide family, and vasopressin; biogenic amines (e.g., dopamine, epinephrine, norepinephrine, histamine, glutamate (metabotropic effect), glucagon, acetylcholine (muscarinic effect), and serotonin); chemokines; lipid mediators of inflammation (e.g., prostaglandins, prostanoids, platelet-activating factor, and leukotrienes); and peptide hormones (e.g., calcitonin, C5a anaphylatoxin, follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), neurokinin, thyrotropin-releasing hormone (TRH), and oxytocin). GPCRs that act as receptors for stimuli that have not yet been identified are known as orphan receptors.

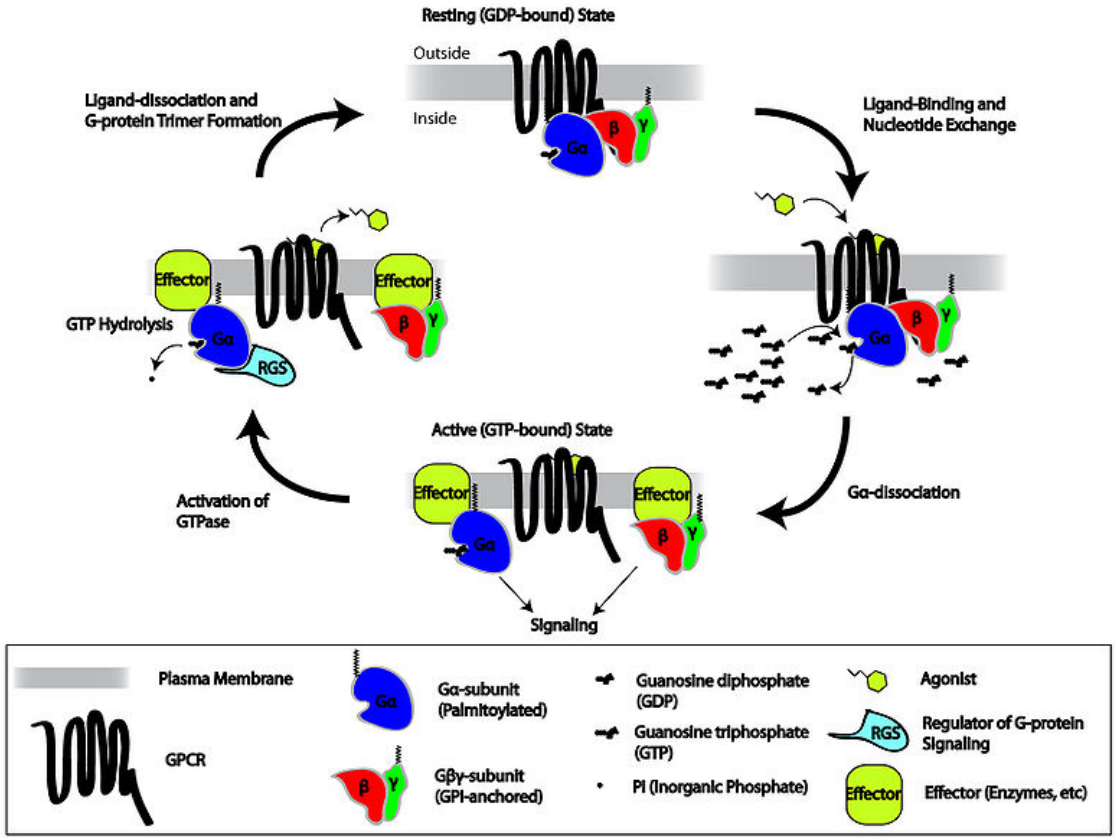
Whereas, in other types of receptors that have been studied, wherein ligands bind externally to the membrane, the ligands of GPCRs typically bind within the transmembrane domain. However, protease-activated receptors are activated by cleavage of part of their extracellular domain.

Conformational change

The transduction of the signal through the membrane by the receptor is not completely understood. It is known that the inactive G protein is bound to the receptor in its inactive state. Once the ligand is recognized, the receptor shifts conformation and, thus, mechanically activates the G protein, which detaches from the receptor. The receptor can now either activate another G protein or switch back to its inactive state. This is an overly simplistic explanation, but suffices to convey the overall set of events.

It is believed that a receptor molecule exists in a conformational equilibrium between active and inactive biophysical states. The binding of ligands to the receptor may shift the equilibrium toward the active receptor states. Three types of ligands exist: Agonists are ligands that shift the equilibrium in favour of active states; inverse agonists are ligands that shift the equilibrium in favour of inactive states; and neutral antagonists are ligands that do not affect the equilibrium. It is not yet known how exactly the active and inactive states differ from each other.

G-protein activation/deactivation cycle

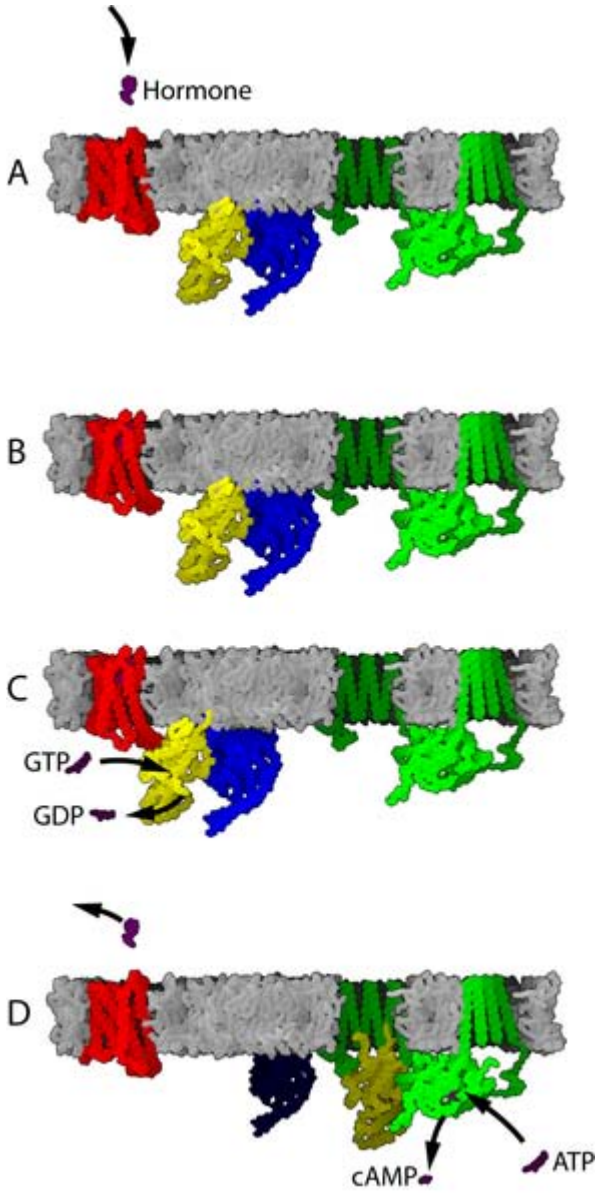


Cartoon depicting the Heterotrimeric G-protein activation/deactivation cycle in the context of GPCR signaling

When the receptor is inactive, the GEF domain may be bound to an also inactive α-subunit of a heterotrimeric G-protein. These "G-proteins" are a trimer of α, β, and γ subunits (known as Gα, Gβ, and Gγ, respectively) which is rendered inactive when reversibly bound to Guanosine diphosphate (GDP) (or alternatively, no guanine nucleotide) but active when bound to Guanosine triphosphate (GTP). Upon receptor activation, the GEF domain, in turn, allosterically activates the G-protein by facilitating the exchange of a molecule of GDP for GTP at the G-protein's α-subunit. The cell maintains a 10:1 ratio of cytosolic GTP:GDP so exchange for GTP is ensured. At this point, the subunits of the G-protein dissociate from the receptor, as well as each other, to yield a Gα-GTP monomer and a tightly interacting Gβγ dimer, which are now free to modulate the activity of other intracellular proteins. The extent to which they may diffuse, however, is limited due to the palmitoylation of Gα and the presence of a molecule of Glycosylphosphatidylinositol (GPI) that has been covalently added to the C-termini of Gγ. The phosphatidylinositol moiety of the GPI-linkage contains two hydrophobic acyl groups that anchor any GPI-linked proteins (e.g. Gβγ) to the plasma membrane, and also, to some extent, to the local lipid raft. (Compare this to the effect of palmitoylation on GPCR localization discussed above)

Because Gα also has slow GTP→GDP hydrolysis capability, the inactive form of the α-subunit (Gα-GDP) is eventually regenerated, thus allowing reassociation with a Gβγ dimer to form the "resting" G-protein which can again bind to a GPCR and await activation. The rate of GTP hydrolysis is often accelerated due to the actions of another family of allosteric modulating proteins called Regulators of G-protein Signaling, or RGS proteins, which are a type of GTPase-Activating Protein, or GAP. In fact, many of the primary effector proteins (e.g. adenylate cyclases) that become activated/inactivated upon interaction with Gα-GTP also have GAP activity. Thus, even at this early stage in the process, GPCR-initiated signaling has the capacity for self-termination.

GPCR signaling



G-protein-coupled receptor mechanism

If a receptor in an active state encounters a G protein, it may activate it. Some evidence suggests that receptors and G proteins are actually pre-coupled. For example, binding of G proteins to receptors affects the receptor's affinity for ligands. Activated G proteins are bound to GTP.

Further signal transduction depends on the type of G protein. The enzyme adenylate cyclase is an example of a cellular protein that can be regulated by a G protein, in this case the G protein G_s. Adenylate cyclase activity is activated when it binds to a subunit of the activated G protein. Activation of adenylate cyclase ends when the G protein returns to the GDP-bound state.

Adenylate cyclases (of which 9 membrane bound and one cytosolic forms are known in humans) may also be activated or inhibited in other ways (e.g. Ca2+/Calmodulin binding) and that this can modify the activity of these enzymes in an additive or synergistic fashion along with the G proteins.

The signaling pathways activated through a GPCR are limited by the primary sequence and tertiary structure of the GPCR itself but ultimately determined by the particular conformation stabilized by a particular ligand, as well as the availability of transducer molecules. Currently, GPCRs are considered to utilize two primary types of transducers: G-proteins and β-arrestins. Because β-arr's only have high affinity to the phosphorylated form of most GPCRs, the majority of signaling is ultimately dependent upon G-protein activation. However, the possibility for interaction does allow for G-protein independent signaling to occur.

G-protein-dependent signaling

There are three main G-protein-mediated signaling pathways, mediated by four sub-classes of G-proteins distinguished from each other by sequence homology ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, and $G_{\alpha 12/13}$). Each sub-class of G-protein consists of multiple proteins, each the product of multiple genes and/or splice variations that may imbue them with differences ranging from subtle to distinct with regard to signaling properties, but in general they appear to be reasonably grouped into four classes. Because the signal transducing properties of the various possible βγ combinations do not appear to radically differ from one another, these classes are defined according to the isoform of their α-subunit.

While most GPCRs are capable of activating more than one Gα-subtype, they also show a preference for one subtype over another. When the subtype activated depends on the ligand that is bound to the GPCR, this is called functional selectivity (also known as agonist-directed trafficking, or conformation specific agonism). However, the binding of any single particular agonist may also initiate activation of multiple different G-proteins, as it may be capable of stabilizing more than one conformation of the GPCR's GEF domain, even over the course of a single interaction. Additionally, a conformation that preferably activates one isoform of Gα may activate another if the preferred is less available. Furthermore, feedback pathways may result in receptor modifications (e.g. phosphorylation) that alter the G-protein preference. Regardless of these various nuances,

the GPCR's preferred coupling partner is usually defined according to the G-protein most obviously activated by the endogenous ligand under most physiological and/or experimental conditions.

G_{α} signaling

1. The effector of both the $G_{\alpha s}$ and $G_{\alpha i/o}$ pathways is the Cyclic-adenosine monophosphate (cAMP) generating enzyme Adenylate Cyclase, or AC. While there are ten different AC gene products in mammals, each with subtle differences in tissue distribution and/or function, all catalyze the conversion of cytosolic Adenosine Triphosphate (ATP) to cAMP, and all are directly stimulated by G-proteins of the $G_{\alpha s}$ class. Conversely, interaction with Gα subunits of the $G_{\alpha i/o}$ type inhibits AC from generating cAMP. Thus, a GPCR coupled to $G_{\alpha s}$ will counteract the actions of a GPCR coupled to $G_{\alpha i/o}$, and vice versa. The level of cytosolic cAMP may then determine the activity of various ion channels as well as members of the ser/thr specific Protein Kinase A (PKA) family. Thus cAMP is considered a second messenger and PKA a secondary effector.
2. The effector of the $G_{\alpha q/11}$ pathway is Phospholipase C-β (PLCβ), which catalyzes the cleavage of membrane-bound phosphatidylinositol 4,5-biphosphate (PIP2) into the second messengers inositol (1,4,5) triphosphate (IP3) and diacylglycerol (DAG). IP3 acts on IP3 receptors found in the membrane of the endoplasmic reticulum (ER) to elicit Ca^{2+} release from the ER, while DAG diffuses along the plasma membrane where it may activate any membrane localized forms of a second ser/thr kinase called Protein Kinase C (PKC). Since many isoforms of PKC are also activated by increases in intracellular Ca^{2+}, both these pathways can also converge on each other to signal through the same secondary effector. Elevated intracellular Ca^{2+} also binds and allosterically activates proteins called Calmodulins, which in turn go on to bind and allosterically activate enzymes such as Ca2+/Calmodulin-dependant Kinases (CAMKs).
3. The effectors of the $G_{\alpha 12/13}$ pathway are three RhoGEFs (p115-RhoGEF, PDZ-RhoGEF, and LARG), which, when bound to $G_{\alpha 12/13}$ allosterically activate the cytosolic small GTPase, Rho. Once bound to GTP, Rho can then go on to activate various proteins responsible for cytoskeleton regulation such as Rho-kinase (ROCK). Most GPCRs that couple to $G_{\alpha 12/13}$ also couple to other sub-classes, often $G_{\alpha q/11}$.

Gβγ signaling

The above descriptions ignore the effects of Gβγ–signalling, which can also be important, particularly in the case of activated $G_{\alpha i/o}$-coupled GPCRs. The primary effectors of Gβγ are various ion channels, such as G-protein-regulated Inwardly Rectifying K+ channels (GIRKs), P/Q- and N-type voltage-gated Ca^{2+} Channels, as well as some isoforms of AC and PLC, along with some Phosphoinositide-3-Kinase (PI3K) isoforms.

G-Protein-independent signaling

Although they are classically thought of working only together, GPCRs may signal through G-protein-independent mechanisms, and heterotrimeric G-proteins may play functional roles independent of GPCRs. GPCRs may signal independently through many proteins already mentioned for their roles in G-protein-dependent signaling such as β-arrs, GRKs, and Srcs. Additionally, further scaffolding proteins involved in subcellular localization of GPCRs (e.g. PDZ-domain-containing proteins) may also act as signal transducers. Most often the effector is a member of the MAPK family.

Examples

In the late 1990s, evidence began accumulating to suggest that some GPCRs are able to signal without G proteins. The ERK2 mitogen-activated protein kinase, a key signal transduction mediator downstream of receptor activation in many pathways, has been shown to be activated in response to cAMP-mediated receptor activation in the slime mold *D. discoideum* despite the absence of the associated G protein α- and β-subunits.

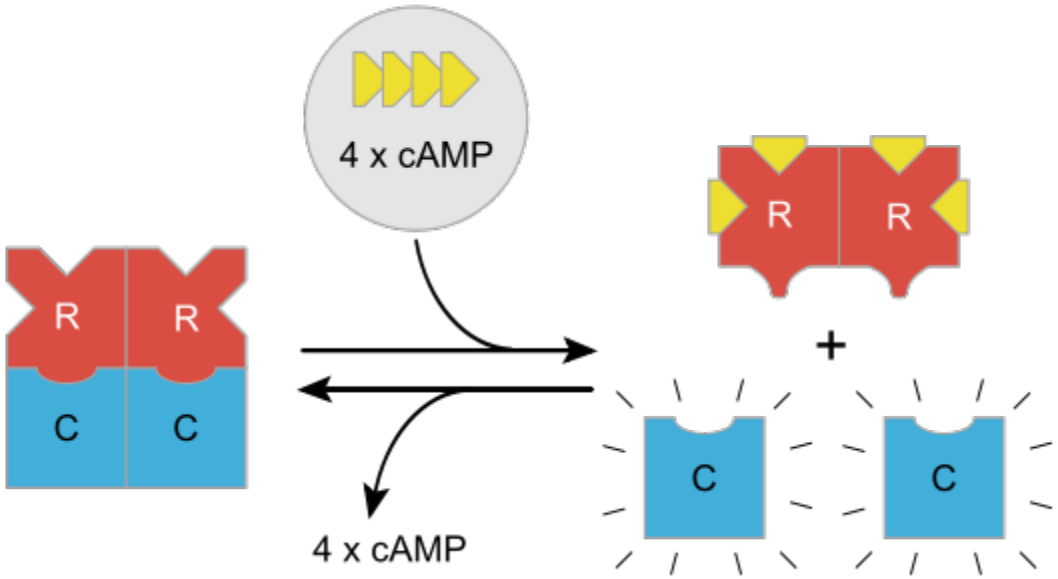
In mammalian cells, the much-studied β_2-adrenoceptor has been demonstrated to activate the ERK2 pathway after arrestin-mediated uncoupling of G-protein-mediated signaling. Therefore it seems likely that some mechanisms previously believed to be purely related to receptor desensitisation are actually examples of receptors switching their signaling pathway rather than simply being switched off.

In kidney cells, the bradykinin receptor B2 has been shown to interact directly with a protein tyrosine phosphatase. The presence of a tyrosine-phosphorylated ITIM (immunoreceptor tyrosine-based inhibitory motif) sequence in the B2 receptor is necessary to mediate this interaction and subsequently the antiproliferative effect of bradykinin.

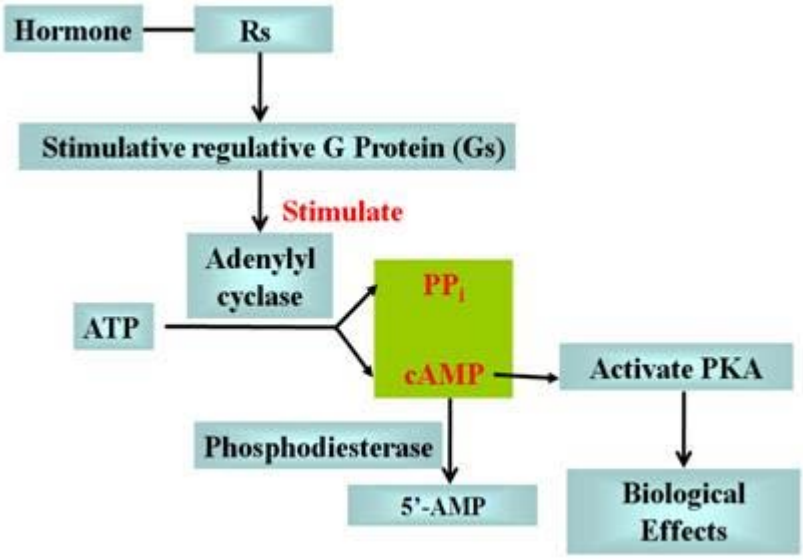
GPCR-independent signaling by heterotrimeric G-proteins

Although it is a relatively immature area of research, it appears that heterotrimeric G-proteins may also take part in non-GPCR signaling. There is evidence for roles as signal transducers in nearly all other types of receptor-mediated signaling, including integrins, receptor tyrosine kinases (RTKs), cytokine receptors (JAK/STATs), as well as modulation of various other "accessory" proteins such as GEFs, Guanine-nucleotide Dissociation Inhibitors (GDIs) and protein phosphatases. There may even be specific proteins of these classes whose primary function is as part of GPCR-independent pathways, termed Activators of G-protein Signalling (AGS). Both the ubiquity of these interactions and the importance of Gα vs. Gβγ subunits to these processes are still unclear.

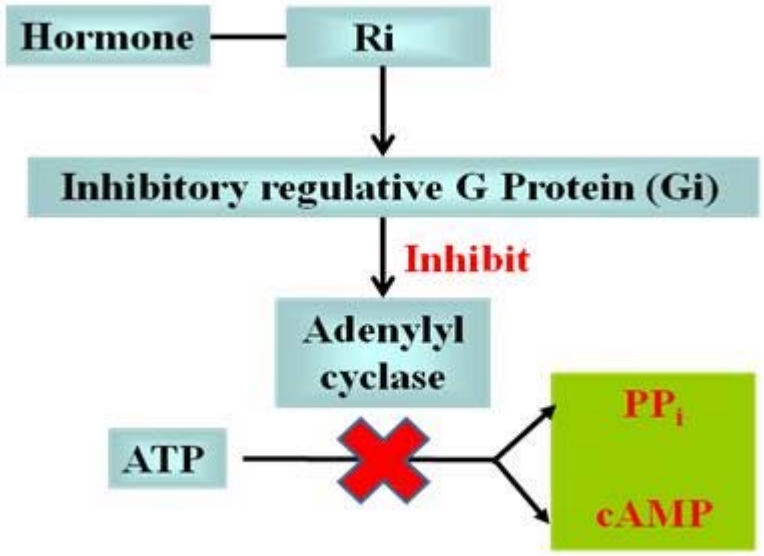
Details of cAMP and PIP2 pathways



Activation effects of cAMP on Protein Kinase A



The effect of Rs and Gs in cAMP signal pathway



The effect of Ri and Gi in cAMP signal pathway

There are two principal signal transduction pathways involving the G protein-linked receptors: cAMP signal pathway and Phosphatidylinositol signal pathway.

cAMP signal pathway

The cAMP signal transduction contains 5 main characters: stimulative hormone receptor (Rs) or inhibitory hormone receptor (Ri) ; Stimulative regulative G-protein (Gs) or inhibitory regulative G-protein (Gi) ; Adenylyl cyclase; Protein Kinase A (PKA); and cAMP phosphodiesterase.

Stimulative hormone receptor (Rs) is a receptor that can bind with stimulative signal molecules, while inhibitory hormone (Ri) is a receptor that can bind with inhibitory signal molecules.

Stimulative regulative G-protein is a G protein-linked to stimulative hormone receptor (Rs) and its α subunit upon activation could stimulate the activity of an enzyme or other intracellular metabolism. On the contrary, inhibitory regulative G-protein is linked to an inhibitory hormone receptor and its α subunit upon activation could inhibit the activity of an enzyme or other intracellular metabolism.

The Adenylyl cyclase is a 12-transmembrane glucoprotein that catalyzes ATP to form cAMP with the help of cofactor Mg^{2+} or Mn^{2+}. The cAMP produced is a second messenger in cellular metabolism and is an allosteric activator to Protein kinase A.

Protein kinase A is an important enzyme in cell metabolism due to its ability to regulate cell metabolism by phosphorylating specific committed enzymes in the metabolic pathway. It can also regulate specific gene expression, cellular secretion, and membrane

permeability. The protein enzyme contains two catalytic subunits and two regulatory subunits. When there is no cAMP, the complex is inactive. When cAMP binds to the regulatory subunits, their conformation is altered, causing the dissociation of the regulatory subunits, which activates protein kinase A and allows further biological effects.

cAMP phosphodiesterase is an enzyme that can degrade cAMP to 5'-AMP, which will terminate the signal.

Phosphatidylinositol signal pathway

In the phosphatidylinositol signal pathway, the extracellular signal molecule binds with the G-protein receptor (G_q) on the cell surface and activates phospholipase C, which is located on the plasma membrane. The lipase hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers: Inositol 1,4,5-triphosphate (IP3) and Diacylglycerol (DAG). IP3 binds with the receptor in the membrane of the smooth endoplasmic reticulum and mitochondria, help open the Ca^{2+} channel. DAG will help activate Protein Kinase C (PKC), which phosphorylates many other proteins, changing their catalytic activities, leading to cellular responses. The effects of Ca^{2+} is also remarkable: it cooperates with DAG in activating PKC and can activate CaM kinase pathway, in which calcium modulated protein calmodulin (CaM) binds Ca^{2+}, undergoes a change in conformation, and activates CaM kinase II, which has unique ability to increase its binding affinity to CaM by autophosphorylation, making CaM unavailable for the activation of other enzymes. The kinase then phosphorylates target enzymes, regulating their activities. The two signal pathways are connected together by Ca^{2+}-CaM, which is also a regulatory subunit of adenylyl cyclase and phosphodiesterase in cAMP signal pathway.

Receptor regulation

GPCRs become desensitized when exposed to their ligand for a prolonged period of time. There are two recognized forms of desensitization: 1) homologous desensitization, in which the activated GPCR is downregulated; and 2) heterologous desensitization, wherein the activated GPCR causes downregulation of a different GPCR. The key reaction of this downregulation is the phosphorylation of the intracellular (or cytoplasmic) receptor domain by protein kinases.

Phosphorylation by cAMP-dependent protein kinases

Cyclic AMP-dependent protein kinases (protein kinase A) are activated by the signal chain coming from the G protein (that was activated by the receptor) via adenylate cyclase and cyclic AMP (cAMP). In a *feedback mechanism*, these activated kinases phosphorylate the receptor. The longer the receptor remains active, the more kinases are activated, the more receptors are phosphorylated. In β_2-adrenoceptors, this phosphorylation results in the switching of the coupling from the G_s class of G-protein to

the G_i class. cAMP-dependent PKA mediated phosphorylation can cause heterologous desensitisation in receptors other than those activated.

Phosphorylation by GRKs

The G protein-coupled receptor kinases (GRKs) are protein kinases that phosphorylate only active GPCRs.

Phosphorylation of the receptor can have two consequences:

1. *Translocation*: The receptor is, along with the part of the membrane it is embedded in, brought to the inside of the cell, where it is dephosphorylated within the acidic vesicular environment and then brought back. This mechanism is used to regulate long-term exposure, for example, to a hormone, by allowing resensitisation to follow desensitisation. Alternatively, the receptor may undergo lysozomal degradation, or remain internalised, where it is thought to participate in the initiation of signalling events, the nature of which depend on the internalised vesicle's subcellular localisation.
2. *Arrestin linking*: The phosphorylated receptor can be linked to *arrestin* molecules that prevent it from binding (and activating) G proteins, effectively switching it off for a short period of time. This mechanism is used, for example, with rhodopsin in retina cells to compensate for exposure to bright light. In many cases, arrestin binding to the receptor is a prerequisite for translocation. For example, beta-arrestin bound to β_2-adrenoreceptors acts as an adaptor for binding with clathrin, and with the beta-subunit of AP2 (clathrin adaptor molecules); thus the arrestin here acts as a scaffold assembling the components needed for clathrin-mediated endocytosis of β_2-adrenoreceptors.

Mechanisms of GPCR signal termination

As mentioned above, G-proteins may terminate their own activation due to their intrinsic GTP→GDP hydrolysis capability. However, this reaction proceeds at a slow rate (≈.02 times/sec) and thus it would take around 50 seconds for any single G-protein to deactivate if other factors did not come into play. Indeed, there are around 30 isoforms of RGS proteins that, when bound to Gα through their GAP domain, accelerate the hydrolysis rate to ≈30 times/sec. This 1500-fold increase in rate allows for the cell to respond to external signals with high speed, as well as spatial resolution due to limited amount of second messenger that can be generated and limited distance a G-protein can diffuse in .03 seconds. For the most part, the RGS proteins are promiscuous in their ability to activate G-proteins, while which RGS is involved in a given signaling pathway seems to be more determined by the tissue and GPCR involved than anything else. Additionally, RGS proteins have the additional function of increasing the rate of GTP-GDP exchange at GPCRs, (i.e. as a sort of co-GEF) further contributing to the time resolution of GPCR signaling.

In addition, the GPCR may be desensitized itself. This can occur as:

1. a direct result of ligand occupation, wherein the change in conformation allows recruitment of GPCR-Regulating Kinases (GRKs), which go on to phosphorylate various serine/threonine residues of IL-3 and the C-terminal tail. Upon GRK phosphorylation, the GPCR's affinity for β-arrestin (β-arrestin-1/2 in most tissues) is increased, at which point β-arrestin may bind and act to both sterically hinder G-protein coupling as well as initiate the process of receptor internalization through clathrin-mediated endocytosis. Because only the liganded receptor is desensitized by this mechanism, it is called homologous desensitization.
2. Alternatively, the affinity for β-arr may increased in a ligand occupation and GRK-independent manner through phosphorylation of different ser/thr sites (but also of IL-3 and the C-terminal tail) by PKC and PKA. These phosphorylations are often sufficient to impair G-protein coupling on their own as well.
3. Thirdly, PKC/PKA may instead phosphorylate GRKs which can also lead to GPCR phosphorylation and β-arrestin binding in an occupation-independent manner. These latter two mechanisms allow for desensitization of one GPCR due to the activities of others, or heterologous desensitization. GRKs may also have GAP domains and so may contribute to inactivation through non-kinase mechanisms as well. A combination of these mechanisms may also occur.

Once β-arrestin is bound to a GPCR it undergoes a conformational change allowing it to serve as a scaffolding protein for an adaptor complex termed AP-2, which in turn recruits another protein called clathrin. If enough receptors in the local area recruit clathrin in this manner, they aggregate and the membrane buds inwardly as a result of interactions between the molecules of clathrin, in a process called opsonization. Once the pit has been pinched off the plasma membrane due to the actions of two other proteins called amphiphysin and dynamin, it is now an endocytic vesicle. At this point the adapter molecules and clathrin have dissociated and the receptor will be trafficked either back to the plasma membrane or targeted to lysosomes for degradation.

At any point in this process, the β-arrestins may also be recruiting other proteins such as the non-receptor tyrosine kinase (nRTK), c-SRC, which may initiate activation of ERK1/2, or other mitogen-activated protein kinase (MAPK) signaling through, for example, phosphorylation of the small GTP-ase, Ras, or recruit the proteins of the ERK cascade directly (i.e. Raf-1, MEK, ERK-1/2) at which point signaling is initiated due to their close proximity to one another. Another target of c-SRC are the dynamin molecules involved in endocytosis. Dynamins polymerize around the neck of an incoming vesicle, and their phosphorylation by c-SRC provides the energy necessary for the conformational change allowing the final “pinching off” from the membrane.

GPCR downregulation

Receptor desensitization is mediated through a combination phosphorylation, β-arr binding, and endocytosis as described above. Downregulation occurs when endocytosed receptor is embedded in an endosome that is trafficked to merge with an organelle called a lysosome. Because lysosomal membranes are rich in proton pumps, their interiors have low pH (≈4.8 vs. the pH≈7.2 cytosol) which acts to denature the GPCRs. Additionally,

lysosomes contain many degradative enzymes, including proteases, which can only function at such low pH, and so the peptide bonds joining the residues of the GPCR together may be cleaved. Whether or not a given receptor is trafficked to a lysosome, detained in endosomes, or trafficked back to the plasma membrane depends on a variety of factors, including receptor type and magnitude of the signal.

Receptor oligomerization

It is generally accepted that G-protein-coupled receptors can form heteromers such as homo- and heterodimers as well as more complex oligomeric structures, and indeed heterodimerization has been shown to be essential for the function of receptors such as the metabotropic GABA(B) receptors. However, it is presently unproven that true heterodimers exist. Present biochemical and physical techniques lack the resolution to differentiate between distinct homodimers assembled into an oligomer or true 1:1 heterodimers. It is also unclear what the functional significance of oligomerization might be, although it is thought that the phenomenon may contribute to the pharmacological heterogeneity of GPCRs in a manner not previously anticipated. This is an actively-studied area in GPCR research.

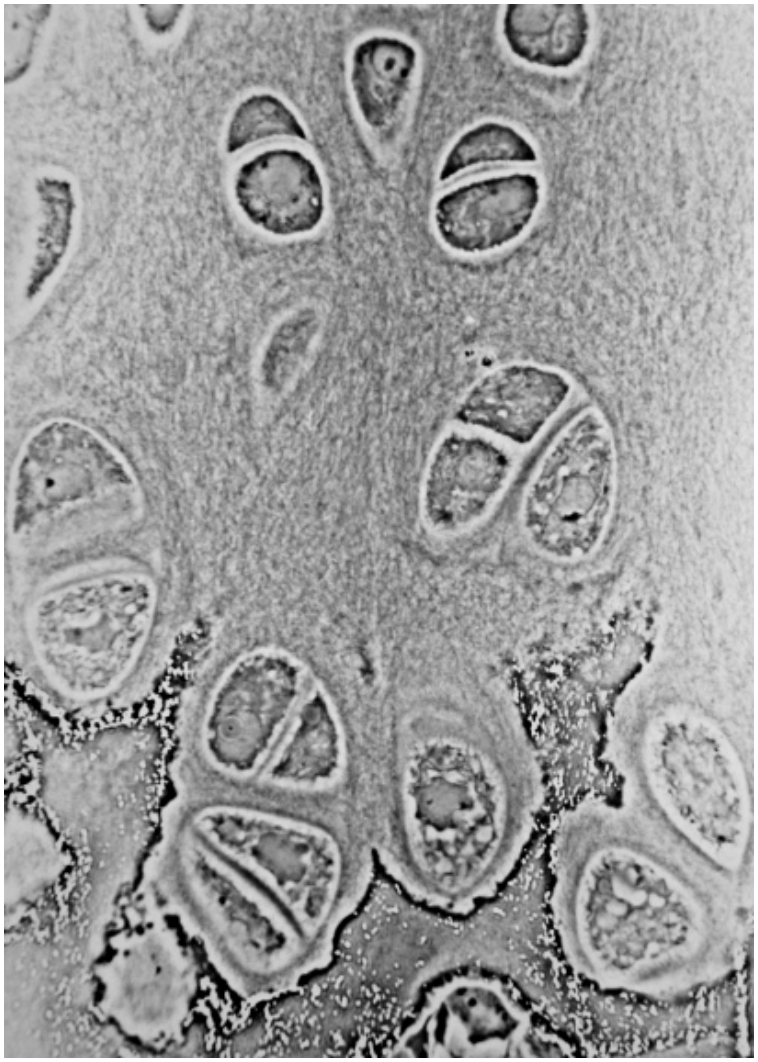
The best-studied example of receptor oligomerisation are the metabotropic $GABA_B$ receptors. These receptors are formed by heterodimerization of $GABA_BR1$ and $GABA_BR2$ subunits. Expression of the $GABA_BR1$ without the $GABA_BR2$ in heterologous systems leads to retention of the subunit in the endoplasmic reticulum. Expression of the $GABA_BR2$ subunit alone, meanwhile, leads to surface expression of the subunit, although with no functional activity (*i.e.*, the receptor does not bind agonist and cannot initiate a response following exposure to agonist). Expression of the two subunits together leads to plasma membrane expression of functional receptor. It has been shown that $GABA_BR2$ binding to $GABA_BR1$ causes masking of a retention signal of functional receptors.

Dictyostelium discoideum

A novel GPCR containing a lipid kinase domain has recently been identified in *Dictyostelium discoideum* that regulates cell density sensing.

Chapter- 4

Apoptosis



Apoptosis increasing from normal cells (top) to apoptotic ones (bottom).

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Apoptosis differs from necrosis, in which the cellular debris can damage the organism.

In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis, in general, confers advantages during an organism's life cycle. For example, the differentiation of fingers and toes in a developing human embryo occurs because cells between the fingers apoptose; the result is that the digits are separate. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult. For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day.

Research in and around apoptosis has increased substantially since the early 1990s. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Excessive apoptosis causes atrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer.

Discovery and etymology

German scientist Carl Vogt was first to describe the principle of apoptosis in 1842. In 1885, anatomist Walther Flemming delivered a more precise description of the process of programmed cell death. However, it was not until 1965 that the topic was resurrected. While studying tissues using electron microscopy, John Foxton Ross Kerr at University of Queensland was able to distinguish apoptosis (Greek: *apo* - from/off/without, *ptosis* - falling) from traumatic cell death. Following the publication of a paper describing the phenomenon, Kerr was invited to join Alastair R Currie, as well as Andrew Wyllie, who was Currie's graduate student, at University of Aberdeen. In 1972, the trio published a seminal article in the British Journal of Cancer. Kerr had initially used the term programmed cell necrosis, but in the article, the process of natural cell death was called *apoptosis*. Kerr, Wyllie and Currie credited James Cormack, a professor of Greek language at University of Aberdeen, with suggesting the term apoptosis. Kerr received the Paul Ehrlich and Ludwig Darmstaedter Prize on March 14, 2000, for his description of apoptosis. He shared the prize with Boston biologist Robert Horvitz.. The 2002 Nobel Prize in Medicine was awarded to Sydney Brenner, Horvitz and John E. Sulston for their work regarding apoptosis.

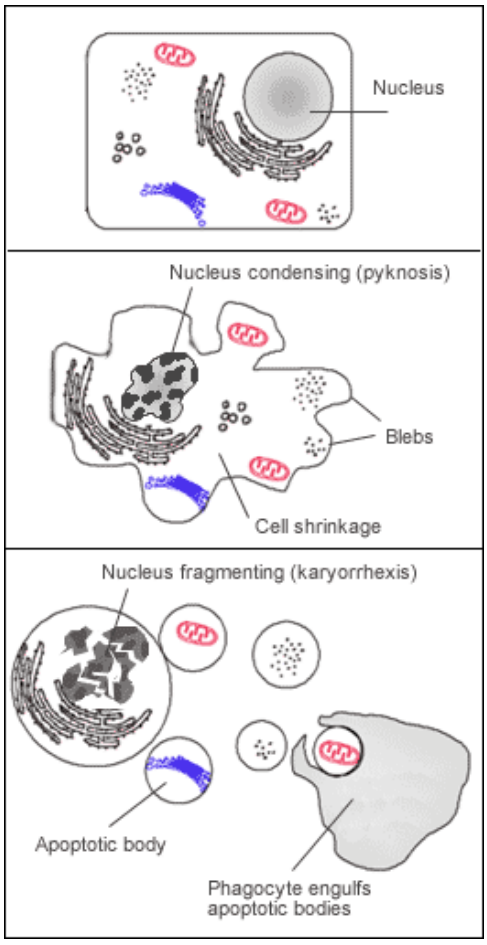
In Greek, apoptosis translates to the "dropping off" of petals or leaves from plants or trees. Cormack, professor of Greek language, reintroduced the term for medical use as it had a medical meaning for the Greeks over two thousand years before. Hippocrates used the term to mean "the falling off of the bones". Galen extended its meaning to "the dropping of the scabs". Cormack was no doubt aware of this usage when he suggested the name. Debate continues over the correct pronunciation, with opinion divided between a

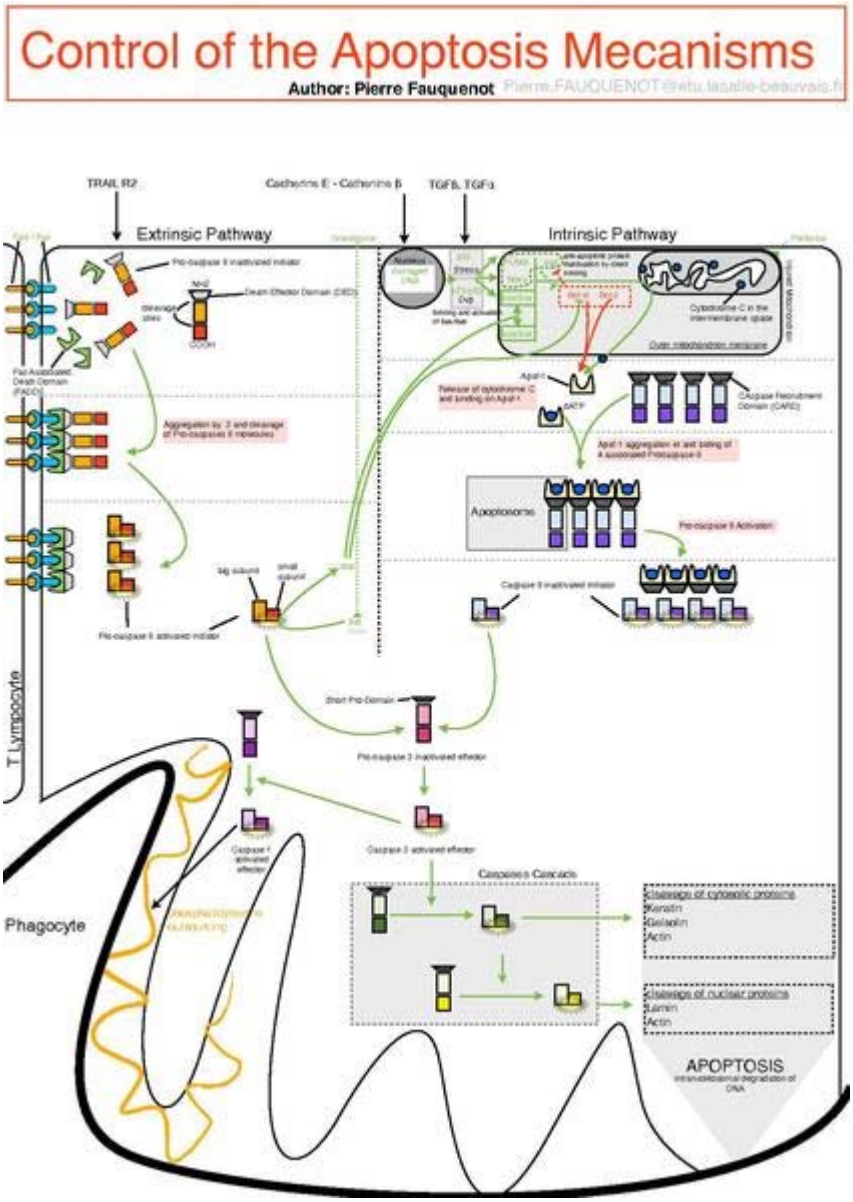
pronunciation with the second *p* silent and the second *p* pronounced, as in the original Greek. In English, the *p* of the Greek *-pt-* consonant cluster is typically silent at the beginning of a word (e.g. pterodactyl, Ptolemy), but articulated when used in combining forms preceded by a vowel, as in helicopter or the orders of insects: diptera, lepidoptera, etc.

In the original Kerr Wyllie and Currie paper, British Journal of Cancer, 1972 Aug;26(4):239-57, there is a footnote regarding the pronunciation:

"We are most grateful to Professor James Cormack of the Department of Greek, University of Aberdeen, for suggesting this term. The word "apoptosis" (Greek spelling of apoptosis) is used in Greek to describe the "dropping off" or "falling off" of petals from flowers, or leaves from trees. To show the derivation clearly, we propose that the stress should be on the penultimate syllable, the second half of the word being pronounced like "ptosis" (with the "p" silent), which comes from the same root "to fall", and is already used to describe the drooping of the upper eyelid."

Process





Control Of The Apoptosis Mecanisms

The process of apoptosis is controlled by a diverse range of cell signals, which may originate either extracellularly (*extrinsic inducers*) or intracellularly (*intrinsic inducers*). Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines, that must either cross the plasma membrane or transduce to effect a response. These signals may positively (i.e., trigger) or negatively (i.e., repress, inhibit, or dampen) affect apoptosis. (Binding and subsequent initiation of apoptosis by a molecule is termed *positive induction*, whereas the active repression or inhibition of apoptosis by a molecule is termed *negative induction*.)

A cell initiates intracellular apoptotic signalling in response to a stress, which may bring about cell suicide. The binding of nuclear receptors by glucocorticoids, heat, radiation,

nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell. A number of cellular components, such as poly ADP ribose polymerase, may also help regulate apoptosis.

Before the actual process of cell death is precipitated by enzymes, apoptotic signals must cause regulatory proteins to initiate the apoptosis pathway. This step allows apoptotic signals to cause cell death, or the process to be stopped, should the cell no longer need to die. Several proteins are involved, but two main methods of regulation have been identified: targeting mitochondria functionality, or directly transducing the signal via adaptor proteins to the apoptotic mechanisms. Another extrinsic pathway for initiation identified in several toxin studies is an increase in calcium concentration within a cell caused by drug activity, which also can cause apoptosis via a calcium binding protease calpain.

Mitochondrial regulation

The mitochondria are essential to multicellular life. Without them, a cell ceases to respire aerobically and quickly dies, a fact exploited by some apoptotic pathways. Apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out. There is also a growing body of evidence indicating that nitric oxide is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria and therefore make it more permeable.

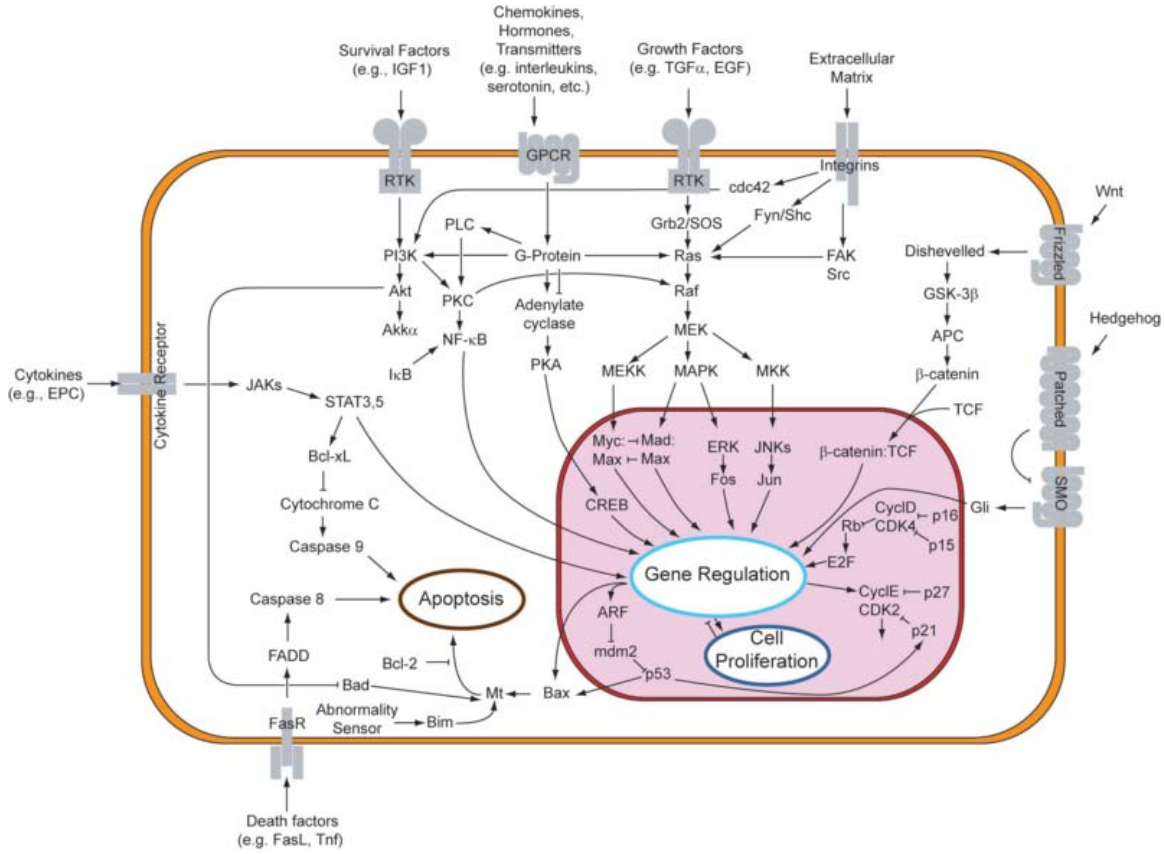
Mitochondrial proteins known as SMACs (second mitochondria-derived activator of caspases) are released into the cytosol following an increase in permeability. SMAC binds to *inhibitor of apoptosis proteins* (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases, which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability.

Cytochrome c is also released from mitochondria due to formation of a channel, MAC, in the outer mitochondrial membrane, and serves a regulatory function as it precedes morphological change associated with apoptosis. Once cytochrome c is released it binds with Apoptotic protease activating factor - 1 (*Apaf-1*) and ATP, which then bind to *pro-caspase-9* to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector *caspase-3*.

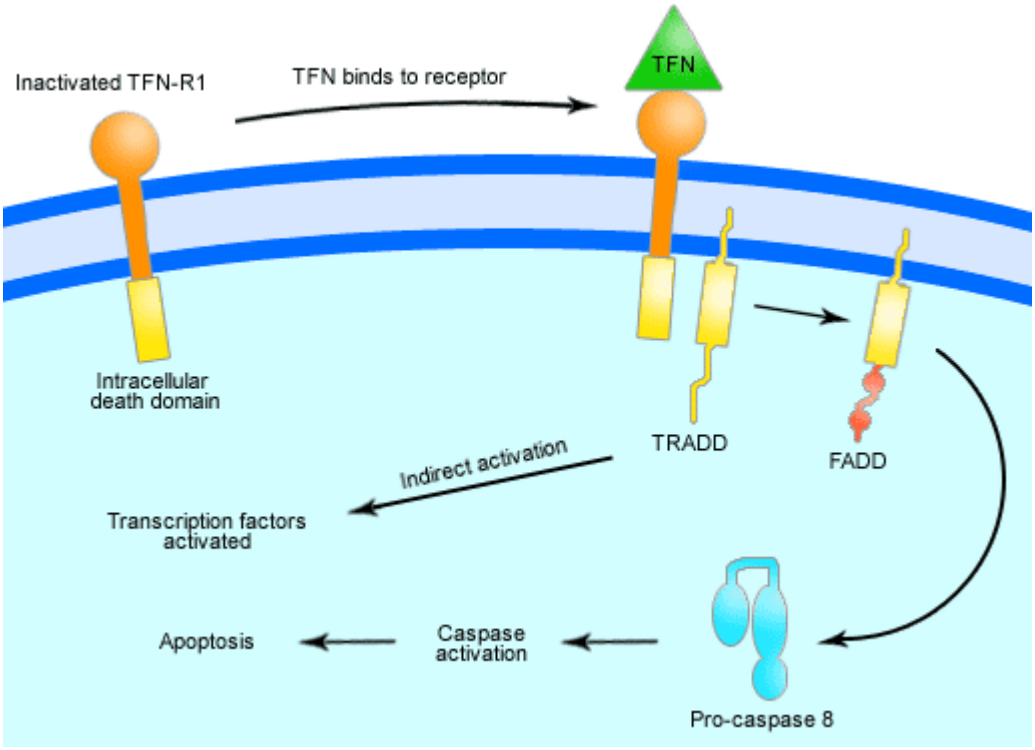
MAC, also called "Mitochondrial Outer Membrane Permeabilization Pore" is regulated by various proteins, such as those encoded by the mammalian *Bcl-2* family of anti-apoptopic genes, the homologs of the *ced-9* gene found in *C. elegans*. *Bcl-2* proteins are

able to promote or inhibit apoptosis by direct action on MAC/MOMPP. Bax and/or Bak form the pore, while Bcl-2, Bcl-xL or Mcl-1 inhibit its formation.

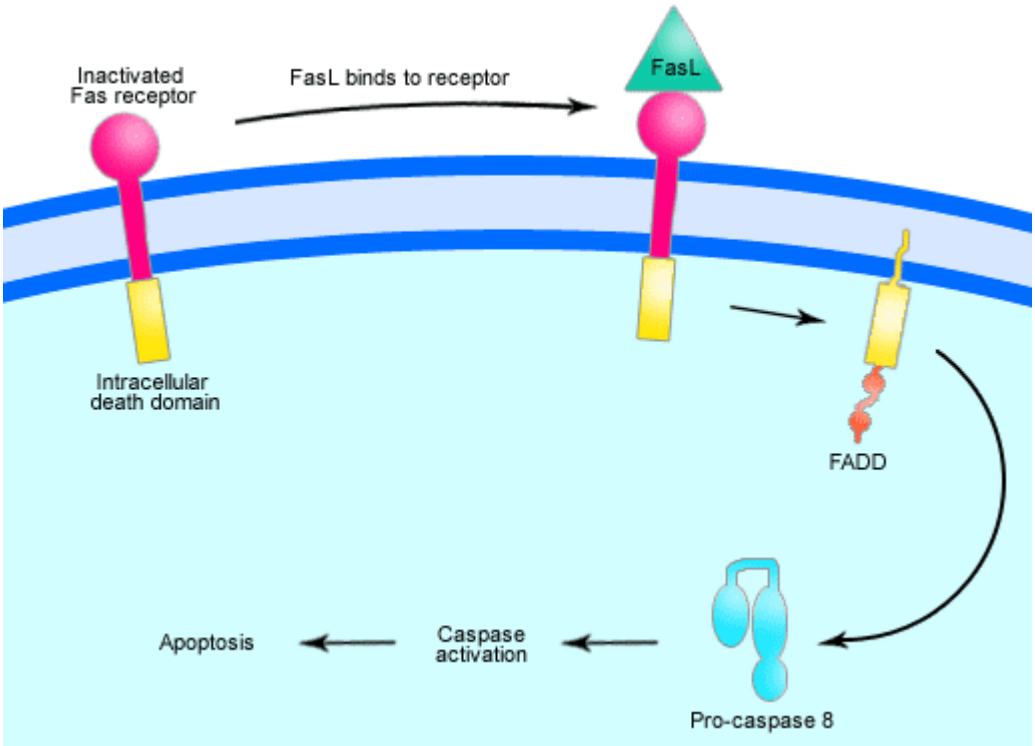
Direct signal transduction



Overview of signal transduction pathways.



Overview of TNF signalling in apoptosis, an example of direct signal transduction



Overview of Fas signalling in apoptosis, an example of direct signal transduction

Two theories of the direct initiation of apoptotic mechanisms in mammals have been suggested: the *TNF-induced* (tumour necrosis factor) model and the *Fas-Fas ligand-mediated* model, both involving receptors of the *TNF receptor* (TNFR) family coupled to extrinsic signals.

TNF path

TNF is a cytokine produced mainly by activated macrophages, and is the major extrinsic mediator of binary hipaloptic apoptosis. Most cells in the human body have two receptors for TNF: *TNF-R1* and *TNF-R2*. The binding of TNF to *TNF-R1* has been shown to initiate the pathway that leads to caspase activation via the intermediate membrane proteins *TNF receptor-associated death domain* (TRADD) and *Fas-associated death domain protein* (FADD). Binding of this receptor can also indirectly lead to the activation of transcription factors involved in cell survival and inflammatory responses. The link between TNF and apoptosis shows why an abnormal production of TNF plays a fundamental role in several human diseases, especially in autoimmune diseases.

Fas path

The Fas receptor (also known as *Apo-1* or *CD95*) binds the Fas ligand (FasL), a transmembrane protein part of the TNF family. The interaction between Fas and FasL results in the formation of the *death-inducing signaling complex* (DISC), which contains the FADD, caspase-8 and caspase-10. In some types of cells (type I), processed caspase-8 directly activates other members of the caspase family, and triggers the execution of apoptosis of the cell. In other types of cells (type II), the *Fas*-DISC starts a feedback loop that spirals into increasing release of pro-apoptotic factors from mitochondria and the amplified activation of caspase-8.

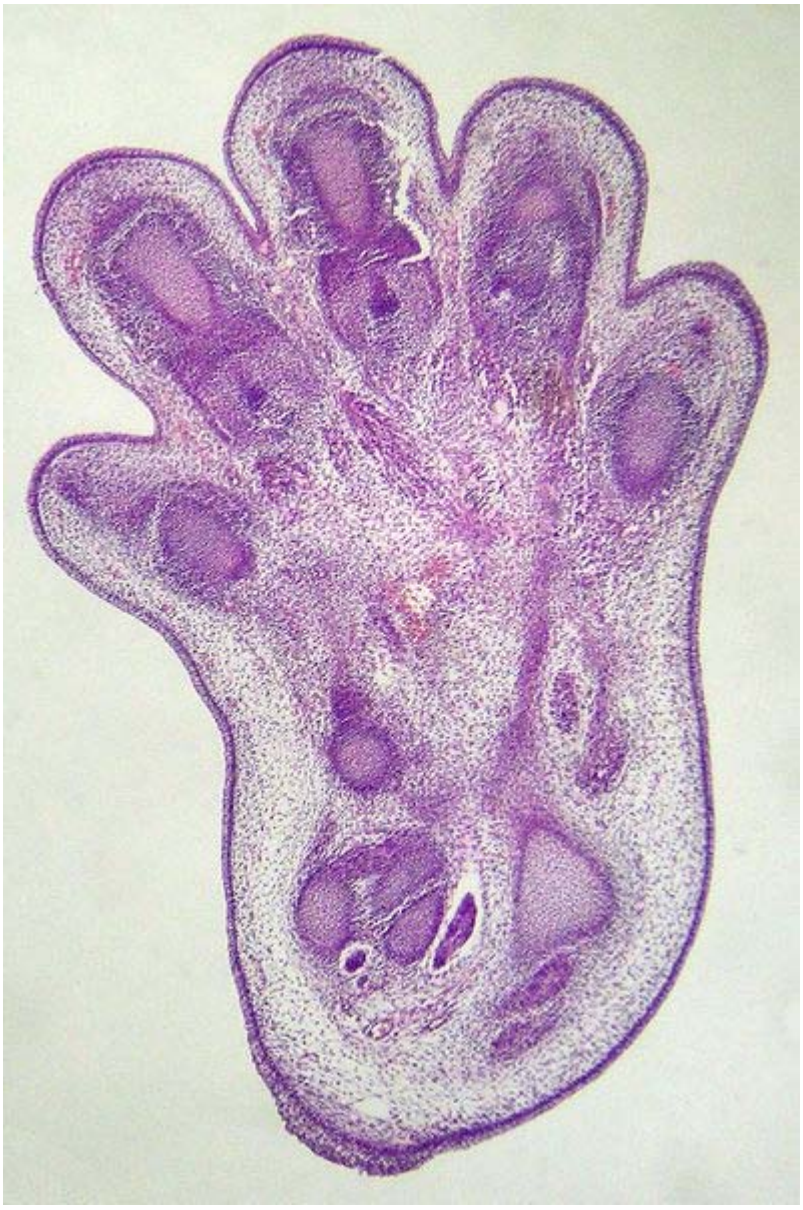
Common components

Following *TNF-R1* and *Fas* activation in mammalian cells a balance between pro-apoptotic (BAX, BID, BAK, or BAD) and anti-apoptotic (*Bcl-Xl* and *Bcl-2*) members of the *Bcl-2* family is established. This balance is the proportion of pro-apoptotic homodimers that form in the outer-membrane of the mitochondrion. The pro-apoptotic homodimers are required to make the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC. Control of pro-apoptotic proteins under normal cell conditions of non-apoptotic cells is incompletely understood, but in general, Bax or Bak are activated by the activation of BH3-only proteins, part of the Bcl-2 family.

Caspase-independent apoptotic pathway

There also exists a caspase-independent apoptotic pathway that is mediated by AIF (apoptosis-inducing factor).

Execution



Histologic cross section of embryonic foot of mouse (*Mus musculus*) in 15.5 day of its development. There are still cells between fingers. (Full development of mouse lasts 27 days.) (Compare this image with image of leg of mouse.)

Many pathways and signals lead to apoptosis, but there is only one mechanism that actually causes the death of a cell. After a cell receives stimulus, it undergoes organized degradation of cellular organelles by activated proteolytic caspases. A cell undergoing apoptosis shows a characteristic morphology:

1. Cell shrinkage and rounding are shown because of the breakdown of the proteinaceous cytoskeleton by caspases.
2. The cytoplasm appears dense, and the organelles appear tightly packed.

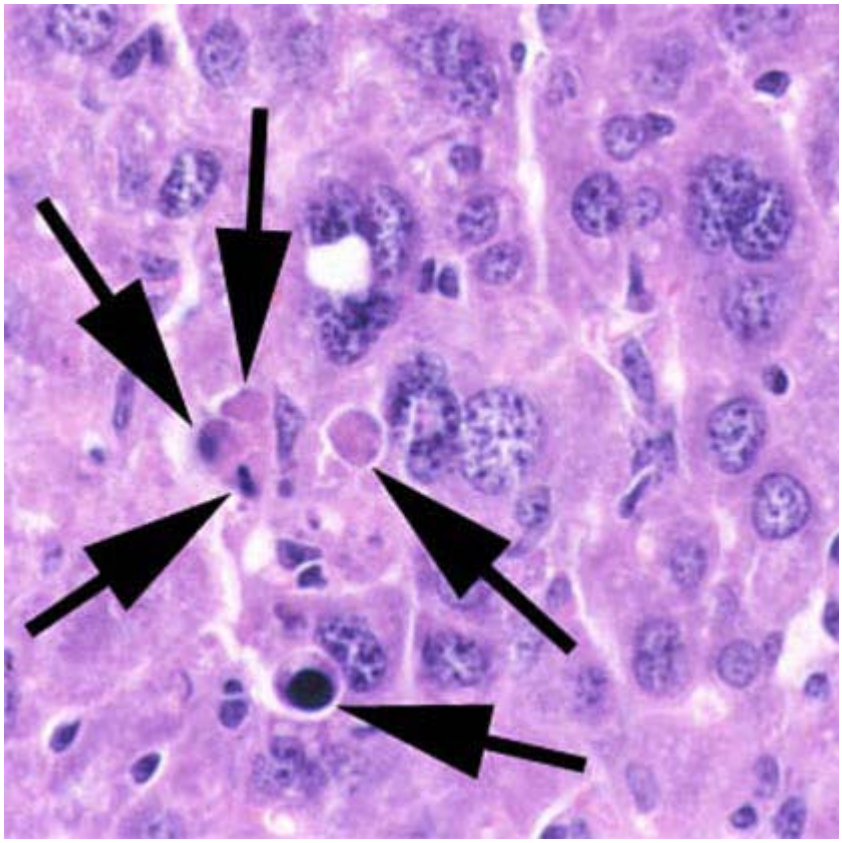
3. Chromatin undergoes condensation into compact patches against the nuclear envelope(also known as the perinuclear envelope) in a process known as pyknosis, a hallmark of apoptosis.
4. The nuclear envelope becomes discontinuous and the DNA inside it is fragmented in a process referred to as karyorrhexis. The nucleus breaks into several discrete *chromatin bodies* or *nucleosomal units* due to the degradation of DNA.
5. The cell membrane shows irregular buds known as blebs.
6. The cell breaks apart into several vesicles called *apoptotic bodies*, which are then phagocytosed.

Apoptosis progresses quickly and its products are quickly removed, making it difficult to detect or visualize. During karyorrhexis, endonuclease activation leaves short DNA fragments, regularly spaced in size. These give a characteristic "laddered" appearance on agar gel after electrophoresis. Tests for DNA laddering differentiate apoptosis from ischemic or toxic cell death.

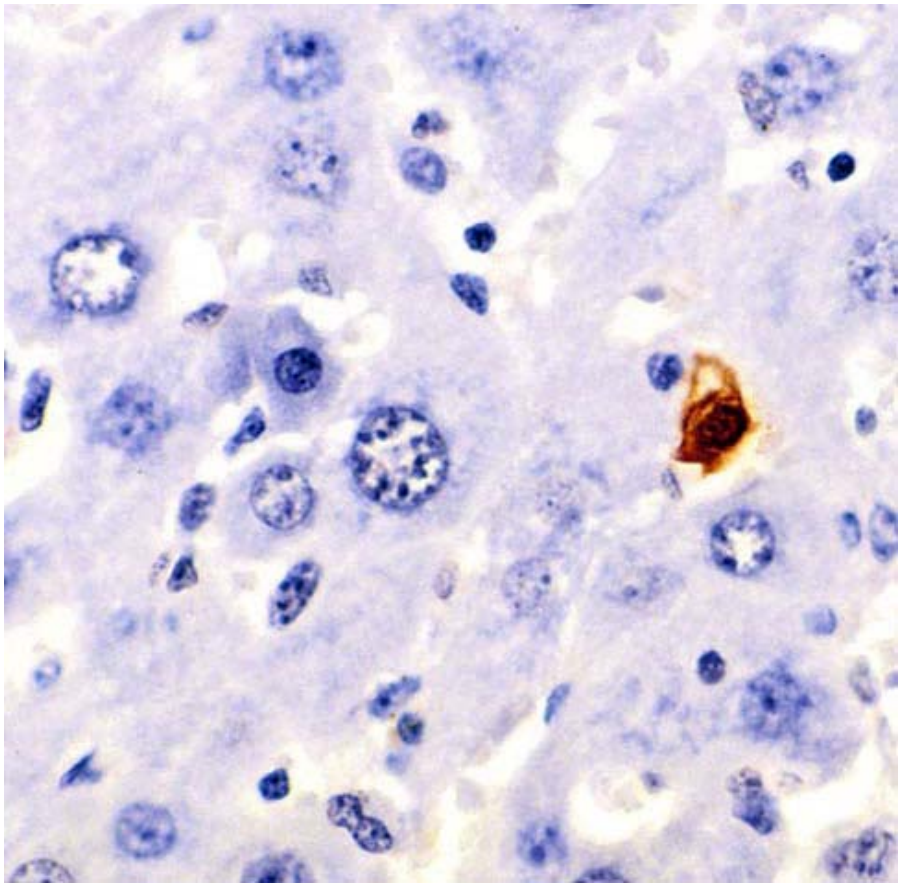
Removal of dead cells

The removal of dead cells by neighboring phagocytic cells has been termed efferocytosis. Dying cells that undergo the final stages of apoptosis display phagocytotic molecules, such as phosphatidylserine, on their cell surface. Phosphatidylserine is normally found on the cytosolic surface of the plasma membrane, but is redistributed during apoptosis to the extracellular surface by a hypothetical protein known as scramblase. These molecules mark the cell for phagocytosis by cells possessing the appropriate receptors, such as macrophages. Upon recognition, the phagocyte reorganizes its cytoskeleton for engulfment of the cell. The removal of dying cells by phagocytes occurs in an orderly manner without eliciting an inflammatory response.

Implication in disease



A section of mouse liver showing several apoptotic cells, indicated by arrows



A section of mouse liver stained to show cells undergoing apoptosis (orange)

Defective apoptotic pathways

The many different types of apoptotic pathways contain a multitude of different biochemical components, many of them not yet understood. As a pathway is more or less sequential in nature, it is a victim of causality; removing or modifying one component leads to an effect in another. In a living organism this can have disastrous effects, often in the form of disease or disorder. A discussion of every disease caused by modification of the various apoptotic pathways would be impractical, but the concept overlying each one is the same: the normal functioning of the pathway has been disrupted in such a way as to impair the ability of the cell to undergo normal apoptosis. This results in a cell that lives past its "use-by-date" and is able to replicate and pass on any faulty machinery to its progeny, increasing the likelihood of the cell becoming cancerous or diseased.

A recently-described example of this concept in action can be seen in the development of a lung cancer called NCI-H460. The *X-linked inhibitor of apoptosis protein* (XIAP) is overexpressed in cells of the H460 cell line. XIAPs bind to the processed form of caspase-9, and suppress the activity of apoptotic activator cytochrome c, therefore overexpression leads to a decrease in the amount of pro-apoptotic agonists. As a consequence, the balance of anti-apoptotic and pro-apoptotic effectors is upset in favour of the former, and the damaged cells continue to replicate despite being directed to die.

Dysregulation of p53

The tumor-suppressor protein p53 accumulates when DNA is damaged due to a chain of biochemical factors. Part of this pathway includes alpha-interferon and beta-interferon, which induce transcription of the *p53* gene and result in the increase of p53 protein level and enhancement of cancer cell-apoptosis. p53 prevents the cell from replicating by stopping the cell cycle at G1, or interphase, to give the cell time to repair, however it will induce apoptosis if damage is extensive and repair efforts fail. Any disruption to the regulation of the *p53* or interferon genes will result in impaired apoptosis and the possible formation of tumors.

HIV progression

The progression of the human immunodeficiency virus infection to AIDS is primarily due to the depletion of CD4+ T-helper lymphocytes, which leads to a compromised immune system. One of the mechanisms by which T-helper cells are depleted is apoptosis, which results from a series of biochemical pathways:

1. HIV enzymes deactivate anti-apoptotic *Bcl-2* This does not directly cause cell death, but primes the cell for apoptosis should the appropriate signal be received. In parallel, these enzymes activate pro-apoptotic *procaspase-8*, which does directly activate the mitochondrial events of apoptosis.
2. HIV may increase the level of cellular proteins which prompt Fas-mediated apoptosis.
3. HIV proteins decrease the amount of CD4 glycoprotein marker present on the cell membrane.
4. Released viral particles and proteins present in extracellular fluid are able to induce apoptosis in nearby "bystander" T helper cells.
5. HIV decreases the production of molecules involved in marking the cell for apoptosis, giving the virus time to replicate and continue releasing apoptotic agents and virions into the surrounding tissue.
6. The infected CD4+ cell may also receive the death signal from a cytotoxic T cell.

Cells may also die as a direct consequence of viral infection. HIV-1 expression induces tubular cell G2/M arrest and apoptosis.

Viral infection

Viruses can trigger apoptosis of infected cells via a range of mechanisms including:

- Receptor binding.
- Activation of protein kinase R (PKR).
- Interaction with p53.
- Expression of viral proteins coupled to MHC proteins on the surface of the infected cell, allowing recognition by cells of the immune system (such as Natural

Killer and cytotoxic T cells) that then induce the infected cell to undergo apoptosis.

Most viruses encode proteins that can inhibit apoptosis. Several viruses encode viral homologs of Bcl-2. These homologs can inhibit pro-apoptotic proteins such as BAX and BAK, which are essential for the activation of apoptosis. Examples of viral Bcl-2 proteins include the Epstein-Barr virus BHRF1 protein and the adenovirus E1B 19K protein. Some viruses express caspase inhibitors that inhibit caspase activity and an example is the CrmA protein of cowpox viruses. Whilst a number of viruses can block the effects of TNF and Fas. For example the M-T2 protein of myxoma viruses can bind TNF preventing it from binding the TNF receptor and inducing a response. Furthermore, many viruses express p53 inhibitors that can bind p53 and inhibit its transcriptional transactivation activity. Consequently p53 cannot induce apoptosis since it cannot induce the expression of pro-apoptotic proteins. The adenovirus E1B-55K protein and the hepatitis B virus HBx protein are examples of viral proteins that can perform such a function.

Interestingly, viruses can remain intact from apoptosis particularly in the latter stages of infection. They can be exported in the *apoptotic bodies* that pinch off from the surface of the dying cell and the fact that they are engulfed by phagocytes prevents the initiation of a host response. This favours the spread of the virus.

Apoptosis in plants

Programmed cell death in plants has a number of molecular similarities to animal apoptosis, but it also has differences, notably the presence of a cell wall and the lack of an immune system which removes the pieces of the dead cell. Instead of an immune response, the dying cell synthesizes substances to break itself down and places them in a vacuole which ruptures as the cell dies. Whether this whole process resembles animal apoptosis closely enough to warrant using the name *apoptosis* (as opposed to the more general *programmed cell death*) is unclear.

Caspase Independent Apoptosis

There is an extrinsic pathway that has been noticed in several toxicity studies. It was shown that an increase in calcium concentration within a cell, caused by drug activity, also has the ability to cause apoptosis via a calcium-binding calpain protease.

Apoptosis Protein Subcellular Location Prediction

In 2003, a method was developed for predicting subcellular location of apoptosis proteins Subsequently, various different modes of Chou's pseudo amino acid composition were developed for improving the quality of predcting subcellular localization of apoptosis proteins based on their sequence information alone.

Chapter- 5

Cytokine

Cytokines (Greek *cyto*-, cell; and -*kinos*, movement) are small cell-signaling protein molecules that are secreted by the glial cells of the nervous system and by numerous cells of the immune system and are a category of signaling molecules used extensively in intercellular communication. Cytokines can be classified as proteins, peptides, or glycoproteins; the term "cytokine" encompasses a large and diverse family of regulators produced throughout the body by cells of diverse embryological origin.

The term "cytokine" has been used to refer to the immunomodulating agents, such as interleukins and interferons. Biochemists disagree as to which molecules should be termed cytokines and which hormones. As we learn more about each, anatomic and structural distinctions between the two are fading. Classic protein hormones circulate in nanomolar (10^{-9}) concentrations that usually vary by less than one order of magnitude. In contrast, some cytokines (such as IL-6) circulate in picomolar (10^{-12}) concentrations that can increase up to 1,000-fold during trauma or infection. The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages (many near the interface with the external environment) are potent producers of IL-1, IL-6, and TNF-α. In contrast, classic hormones, such as insulin, are secreted from discrete glands (e.g., the pancreas). As of 2008, the current terminology refers to cytokines as immunomodulating agents. However, more research is needed in this area of defining cytokines and hormones.

Part of the difficulty with distinguishing cytokines from hormones is that some of the immunomodulating effects of cytokines are systemic rather than local. For instance, to use hormone terminology, the action of cytokines may be autocrine or paracrine in chemotaxis and endocrine as a pyrogen. Further, as molecules, cytokines are not limited to their immunomodulatory role. For instance, cytokines are also involved in several developmental processes during embryogenesis

Effects

Each cytokine has a matching cell-surface receptor. Subsequent cascades of intracellular signalling then alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcription factors, resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition.

The effect of a particular cytokine on a given cell depends on the cytokine, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface, and downstream signals activated by receptor binding; these last two factors can vary by cell type. Cytokines are characterized by considerable "redundancy", in that many cytokines appear to share similar functions.

It seems to be a paradox that cytokines binding to antibodies have a stronger immune effect than the cytokine alone. This may lead to lower therapeutic doses.

Said et al. showed that inflammatory cytokines cause an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes which leads to IL-10 production by monocytes after binding of PD-1 by PD-L.

Nomenclature

Cytokines have been classed as lymphokines, interleukins, and chemokines, based on their presumed function, cell of secretion, or target of action. Because cytokines are characterised by considerable redundancy and pleiotropism, such distinctions, allowing for exceptions, are obsolete.

- The term *interleukin* was initially used by researchers for those cytokines whose presumed targets are principally leukocytes. It is now used largely for designation of newer cytokine molecules discovered every day and bears little relation to their presumed function. The vast majority of these are produced by T-helper cells.
- The term *chemokine* refers to a specific class of cytokines that mediates chemoattraction (chemotaxis) between cells.

Classification

Structural

Structural homology has been able to partially distinguish between cytokines that do not demonstrate a considerable degree of redundancy so that they can be classified into four types:

- The four-α-helix bundle family - Member cytokines have three-dimensional structures with four bundles of α-helices. This family, in turn, is divided into three sub-families:

1. the IL-2 subfamily
2. the interferon (IFN) subfamily
3. the IL-10 subfamily.
    - The first of these three subfamilies is the largest. It contains several non-immunological cytokines including erythropoietin (EPO) and thrombopoietin (TPO). Also, four α-helix bundle cytokines can be grouped into *long-chain* and *short-chain* cytokines.
- the IL-1 family, which primarily includes IL-1 and IL-18
- the IL-17 family, which has yet to be completely characterized, though member cytokines have a specific effect in promoting proliferation of T-cells that cause cytotoxic effects

Functional

A classification that proves more useful in clinical and experimental practice divides immunological cytokines into those that enhance cellular immune responses, type 1 (IFN-γ, TGF-β, etc.), and type 2 (IL-4, IL-10, IL-13, etc.), which favor antibody responses.

A key focus of interest has been that cytokines in one of these two sub-sets tend to inhibit the effects of those in the other. Dysregulation of this tendency is under intensive study for its possible role in the pathogenesis of autoimmune disorders.

Several inflammatory cytokines are induced by oxidant stress. The fact that cytokines themselves trigger the release of other cytokines and also lead to increased oxidant stress makes them important in chronic inflammation.

Cytokine receptors

In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics, and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleiomorphism of cytokines are, in fact, a consequence of their homologous receptors, many authorities think that a classification of cytokine receptors would be more clinically and experimentally useful.

A classification of cytokine receptors based on their three-dimensional structure has, therefore, been attempted. Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.

- Immunoglobulin (Ig) superfamily, which are ubiquitously present throughout several cells and tissues of the vertebrate body, and share structural homology with immunoglobulins (antibodies), cell adhesion molecules, and even some cytokines. Examples: IL-1 receptor types.
- Haemopoietic Growth Factor (type 1) family, whose members have certain conserved motifs in their extracellular amino-acid domain. The IL-2 receptor

belongs to this chain, whose γ-chain (common to several other cytokines) deficiency is directly responsible for the x-linked form of Severe Combined Immunodeficiency (X-SCID).
- Interferon (type 2) family, whose members are receptors for IFN β and γ.
- Tumor necrosis factors (TNF) (type 3) family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like CD40, CD27 and CD30, besides the ligands on which the family is named (TNF).
- Seven transmembrane helix family, the ubiquitous receptor type of the animal kingdom. All G protein-coupled receptors (for hormones and neurotransmitters) belong to this family. Chemokine receptors, two of which act as binding proteins for HIV (CXCR4 and CCR5), also belong to this family.

Disease

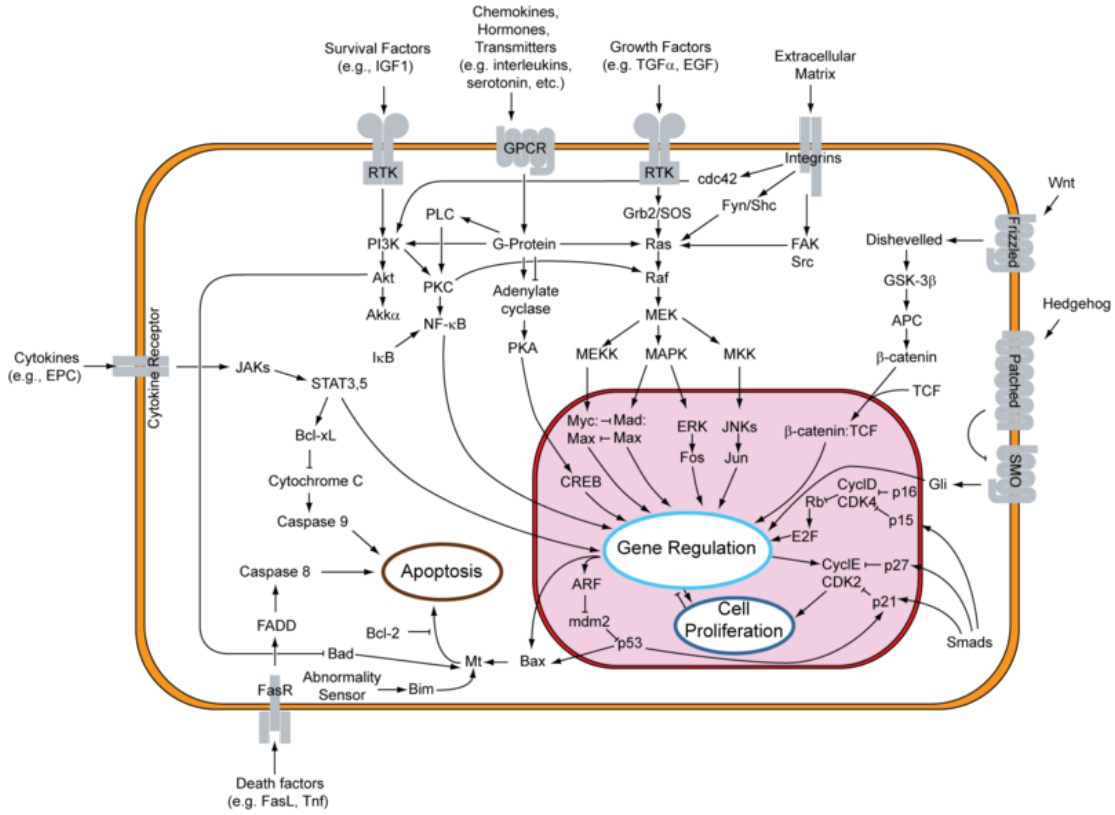
Adverse effects of cytokines have been linked to many disease states and conditions ranging from major depression and Alzheimer's disease to cancer with levels either being elevated or changed. Oversecretion of cytokines can trigger a dangerous syndrome known as a cytokine storm; this may have been the cause of severe adverse events during a clinical trial of TGN1412.

Plasma levels

Plasma levels of various cytokines may give information on the presence, or even predictive value of inflammatory processes involved in autoimmune diseases such as rheumatoid arthritis, as well as immunomodulatory effects of foods or drugs . In addition, elevated levels of IL-7, an important cytokine involved in T cell homeostasis, have been detected in the plasma of HIV-infected patients.

Chapter- 6

Signal Transduction



Overview of signal transduction pathways

Signal transduction comes from the verb to 'transduce' meaning to 'lead across'. In biology **signal transduction** is the process by which an extracellular signaling molecule activates a membrane receptor that in turn alters intracellular molecules to create a response. Transmembrane receptors span the cell membrane, with part of the receptor outside and part inside the cell. The chemical signal binds to the outer portion of the receptor, changing its shape and conveying another signal inside the cell. Some chemical

messengers, such as testosterone, can pass through the cell membrane, and bind directly to receptors in the cytoplasm or nucleus.

Sometimes there is a cascade of signals within the cell. With each step of the cascade, the signal can be amplified, so a small signal can result in a large response. Eventually, the signal creates a change in the cell, either in the expression of the DNA in the nucleus or in the activity of enzymes in the cytoplasm.

These processes can take milliseconds (for ion flux), minutes (for protein- and lipid-mediated kinase cascades), hours, or days (for gene expression).

Signaling molecules

Most signal transduction involves the binding of extracellular signaling molecules (and ligands) to cell-surface receptors. While triggering events inside the cell, such receptors typically face outward from the plasma membrane. Intracellular signaling cascades can also be triggered through cell-substratum interactions. One example is integrins, which bind ligands found within the extracellular matrix. Steroids are another example of extracellular signaling molecules that may cross the plasma membrane due to their lipophilic or hydrophobic nature. Many, but not all, steroid hormones have receptors within the cytoplasm, and usually act by stimulating the binding of their receptors to the promoter region of steroid-responsive genes. Within multicellular organisms, numerous small molecules and polypeptides serve to coordinate a cell's individual biological activity within the context of the organism as a whole. These molecules have been functionally classified as:

- hormones (e.g., melatonin),
- growth factors (e.g. epidermal growth factor),
- extra-cellular matrix components (e.g., fibronectin),
- cytokines (e.g., interferon-gamma),
- chemokines (e.g., RANTES),
- neurotransmitters (e.g., acetylcholine), and
- neurotrophins (e.g., nerve growth factor).
- active oxygen species and other electronically-activated compounds.

Most of these classifications do not take into account the molecular nature of each class member. For example, as a class, neurotransmitters consist of neuropeptides such as endorphins and small molecules such as serotonin and dopamine. Hormones, another generic class of molecules capable of initiating signal transduction, include insulin (a polypeptide), testosterone (a steroid), and epinephrine (an amino acid derivative, in essence a small organic molecule).

The classification of one molecule into one class or another is not exact. For example, epinephrine and norepinephrine, secreted by the central nervous system, act as neurotransmitters. However, when secreted by the adrenal medulla, epinephrine acts as a hormone.

Environmental stimuli

In bacteria and other single-cell organisms, the variety of signal transduction processes possible influences how it reacts and responds to its environment. In multicellular organisms, numerous signal transduction processes are required for coordinating the behavior of individual cells to support the function of the organism as a whole. The complexity of an organism's signal transduction processes tends to increase with the complexity of the organism itself. Sensing of both the external and internal environments at the cellular level relies on signal transduction. Many disease processes, such as diabetes, heart disease, autoimmunity, and cancer arise from defects in signal transduction pathways, further highlighting the critical importance of signal transduction to biology, as well as medicine.

Various environmental stimuli, in addition to many of the regular signal transduction stimuli listed above, initiate signal transmission processes in complex organisms. Environmental stimuli may also be molecular in nature (as above) or more physical, such as light striking cells in the retina of the eye, odorants binding to odorant receptors in the nasal epithelium, and bitter and sweet tastes stimulating taste receptors in the taste buds.

Certain microbial molecules, e.g., viral nucleotides, bacterial lipopolysaccharides, and protein antigens, are able to elicit an immune system response against invading pathogens, mediated by signal transduction processes. An immune response may occur independent of signal transduction stimulation by other molecules, as is the case for signal transduction by way of the Toll-like receptor or with help from stimulatory molecules located at the cell surface of other cells, as is the case for T-cell receptor signaling.

Unicellular organisms may also respond to environmental stimuli through the activation of signal transduction pathways. For example, slime molds secrete cyclic-AMP upon starvation, which stimulates individual cells in the immediate environment to aggregate. Yeast use mating factors to determine the mating types of other yeast and to participate in sexual reproduction.

Cellular responses

Activation of genes, alterations in metabolism, the continued proliferation and death of the cell, and the stimulation or suppression of locomotion, are some of the cellular responses to extracellular stimulation that require signal transduction. Gene activation leads to further cellular effects, since the protein products of many of the responding genes include enzymes and transcription factors themselves. Transcription factors produced as a result of a signal transduction cascade can, in turn, activate yet more genes. Therefore an initial stimulus can trigger the expression of an entire cohort of genes, and this, in turn, can lead to the activation of any number of complex physiological events. These events include the increased uptake of glucose from the blood stream stimulated by insulin and the migration of neutrophils to sites of infection

stimulated by bacterial products. The set of genes and the order in which they are activated in response to stimuli are often referred to as a *genetic program*.

Neurotransmitters are ligands that are capable of binding to ion channel proteins, resulting in their opening to allow the rapid flow of a particular ion across the plasma membrane. This results in an altering of the cell's membrane potential and is important for processes such as the neural conduction of electrochemical impulses. Ligands can be freely soluble, or can be found on the surface of other cells or within the extracellular matrix. Such cell surface or extracellular matrix ligands signal between cells when they come in contact with each other, such as when a phagocytic cell presents antigens to lymphocytes, or upon adhesion to the extracellular matrix, as when integrins at the cell surface of fibroblasts engage fibronectin.

Most mammalian cells require stimulation to control not only cell division but also survival. In the absence of growth factor stimulation, programmed cell death ensues in most cells. Such requirements for extra-cellular stimulation are necessary for controlling cell behavior in the context of both unicellular and multi-cellular organisms. Signal transduction pathways are perceived to be so central to biological processes that it is not surprising that a large number of diseases have been attributed to their disregulation.

Discussed below are how signal transduction via various classes of receptor may lead to the above cellular responses.

Types of receptor

Receptors can be roughly divided into two major classes:

1. Intracellular receptors and
2. Cell-surface receptors.

Ligand-gated ion channel receptors are a class of receptor that may occur both at the cell-surface or intracellularly.

Solely intracellular receptors include those for steroid hormones, thyroid hormone, retinoic acid, and derivatives of vitamin D_3. In contrast to ligands that bind to cell surface receptors to initiate signal transduction, these ligands must cross the cell membrane.

Major categories of intracellular receptors include G-protein linked receptors and Tyrosine Kinase receptors.

Cell-surface receptors

Cell-surface receptors are integral transmembrane proteins and recognize the vast majority of extracellular signaling molecules. Transmembrane receptors span the plasma membrane of the cell, with one part of the receptor on the outside of the cell (the

extracellular domain), and the other on the inside of the cell (the *intracellular domain*). Signal transduction occurs as a result of stimulatory molecule or the binding of a ligand to its extracellular domain; the ligand itself does not pass through the plasma membrane prior to receptor-binding.

Binding of a ligand to a cell-surface receptor stimulates a series of events inside the cell, with different types of receptor stimulating different intracellular responses. Receptors typically respond to only the binding of a specific ligand. Upon binding, the ligand initiates the transmission of a signal across the plasma membrane by inducing a change in the shape or conformation of the intracellular part of the receptor. Often, such changes in conformation either result in the activation of an enzymatic activity contained within the receptor or expose a binding site for other signaling proteins within the cell. Once these proteins bind to the receptor, they themselves may become active and propagate the signal into the cytoplasm.

In eukaryotic cells, most intracellular proteins activated by a ligand/receptor interaction possess an enzymatic activity. These enzymes include tyrosine kinase, heterotrimeric G proteins, small GTPases, various serine/threoine protein kinases, phosphatases, lipid kinases, and hydrolases. Some receptor-stimulated enzymes create specific second messengers including cyclic nucleotides, such as cyclic AMP (cAMP) and cyclic GMP (cGMP), Phosphatidylinositol derivatives, such as Phosphatidylinositol-trisphosphate (PIP_3), Diacylglycerol (DAG) and Inositol-triphosphate (IP_3), IP_3, controlling the release of intracellular calcium stores into the cytoplasm. Other activated proteins interact with adapter proteins. Adapter proteins facilitate interactions between other signaling proteins, and coordinate the formation of signaling complexes necessary to produce an appropriate cellular response to a particular stimulus. Enzymes and adapter proteins are both responsive to various second messenger molecules.

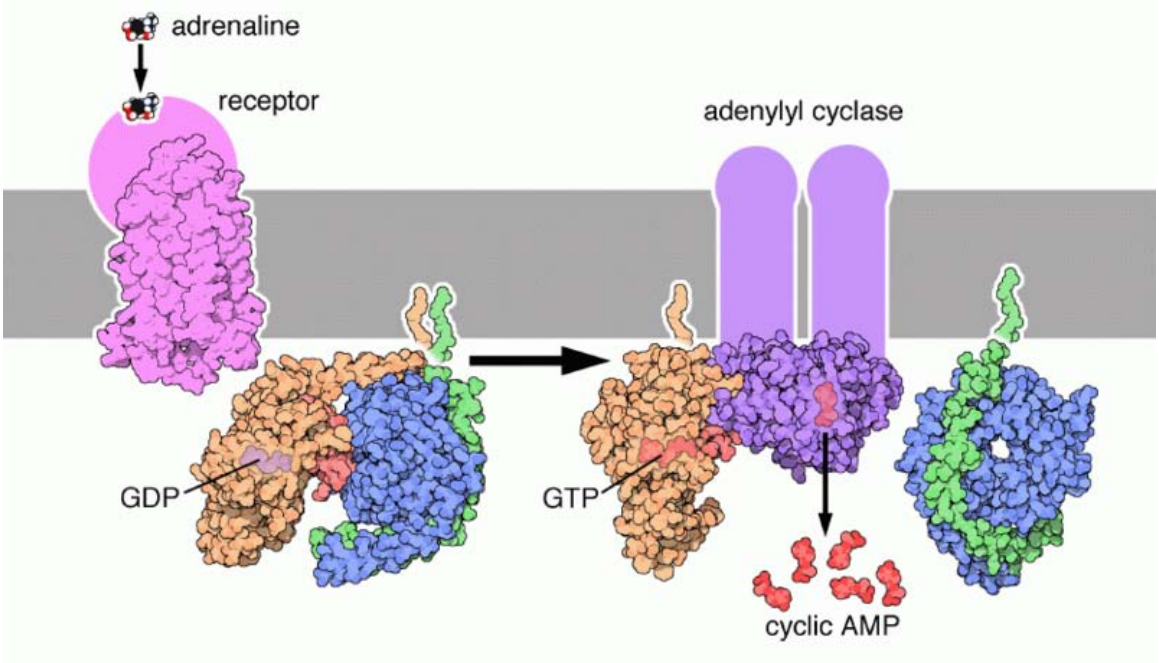
Many of the enzymes activated as part of the signal transduction mechanism and also many adapter proteins have been found to possess specialized protein domains that bind to specific secondary messenger molecules. For example, calcium ions bind specifically to the EF hand domains of calmodulin, allowing this molecule to bind and activate Calmodulin-dependent kinase. PIP_3, PIP_2 and other phosphoinositides may bind to the Pleckstrin homology domains of proteins such as the kinase protein AKT again with activation activity.

There are many different classes of transmembrane receptor that recognize different extracellular signaling molecules. Specific example are:

1. G-protein coupled receptors, e.g., Chemokine receptors
2. Receptor tyrosine kinases, e.g., Growth factor receptors,
3. Integrins
4. Toll-like receptors

Further examples are given in the transmembrane receptor article.

G-protein-coupled receptors



Signal transduction from a G-protein-linked receptor following interaction with its hormone ligand

G-protein-coupled receptors (GPCRs) are a family of integral membrane proteins that possess seven membrane-spanning domains, and are linked to a guanine nucleotide-binding protein (or heterotrimeric G protein). Many receptors make up this family, including adrenergic receptors, neurotransmitter receptors, olfactory receptors, opioid receptors, chemokine receptors, and rhodopsin.

Signal transduction by a GPCR begins with an inactive G protein coupled to the receptor. An inactive G protein exists as a heterotrimer, a molecule composed of three different protein subunits: Gα, Gβ, and Gγ. Once the GPCR recognizes a ligand, the shape (conformation) of the receptor changes to mechanically activate the G protein, and causes one subunit (Gα) to bind a molecule of GTP (causing activation) and dissociate from the other two G-protein subunits (Gβ and Gγ). The dissociation exposes sites on the G-protein subunits that interact with other molecules. The activated G protein subunits detach from the receptor and initiate signaling from many *downstream* effector proteins. These include phosphodiesterases and adenylyl cyclases, phospholipases, and ion channels that permit the release of second messenger molecules such as cyclic-AMP (cAMP), cyclic-GMP (cGMP), inositol triphosphate (IP3), diacylglycerol (DAG), and calcium (Ca^{2+}) ions. For example, a rhodopsin molecule in the plasma membrane of a retina cell in the eye that was activated by a photon can activate up to 2000 effector molecules (in this case, transducin) per second.

The total strength of signal amplification by a GPCR is determined by:

- *The lifetime of the ligand-receptor-complex.* If the ligand-receptor-complex is stable, it takes longer for the ligand to dissociate from its receptor, thus the receptor will remain active for longer and will activate more effector proteins.
- *The amount and lifetime of the receptor-effector protein-complex.* The more effector protein is available to be activated by the receptor, and the faster the activated effector protein can dissociate from the receptor, the more effector protein will be activated in the same amount of time.
- *Deactivation of the activated receptor.* A receptor that is engaged in a hormone-receptor-complex can be deactivated, either by covalent modification (for example, phosphorylation) or by internalization.
- *Deactivation of effectors through intrinsic enzymatic activity.* Either small or large G-proteins possess intrinsic GTPase activity, which controls the duration of the triggered signal. This activity may be increased through the action of other proteins such as GTPase-activating proteins (GAPS).

The idea that G-protein-coupled receptors, to be specific, chemokine receptors, participate in cancer development is suggested by a study wherein a point mutation was inserted into the gene encoding the chemokine receptor CXCR2. Cells transfected with the CXCR2 mutant underwent a malignant transformation. The result of the point mutation was the expression of CXCR2 in an active conformation, despite the absence of chemokine-binding (the CXCR2 mutant is said to be "constitutively active").

Receptor tyrosine kinases

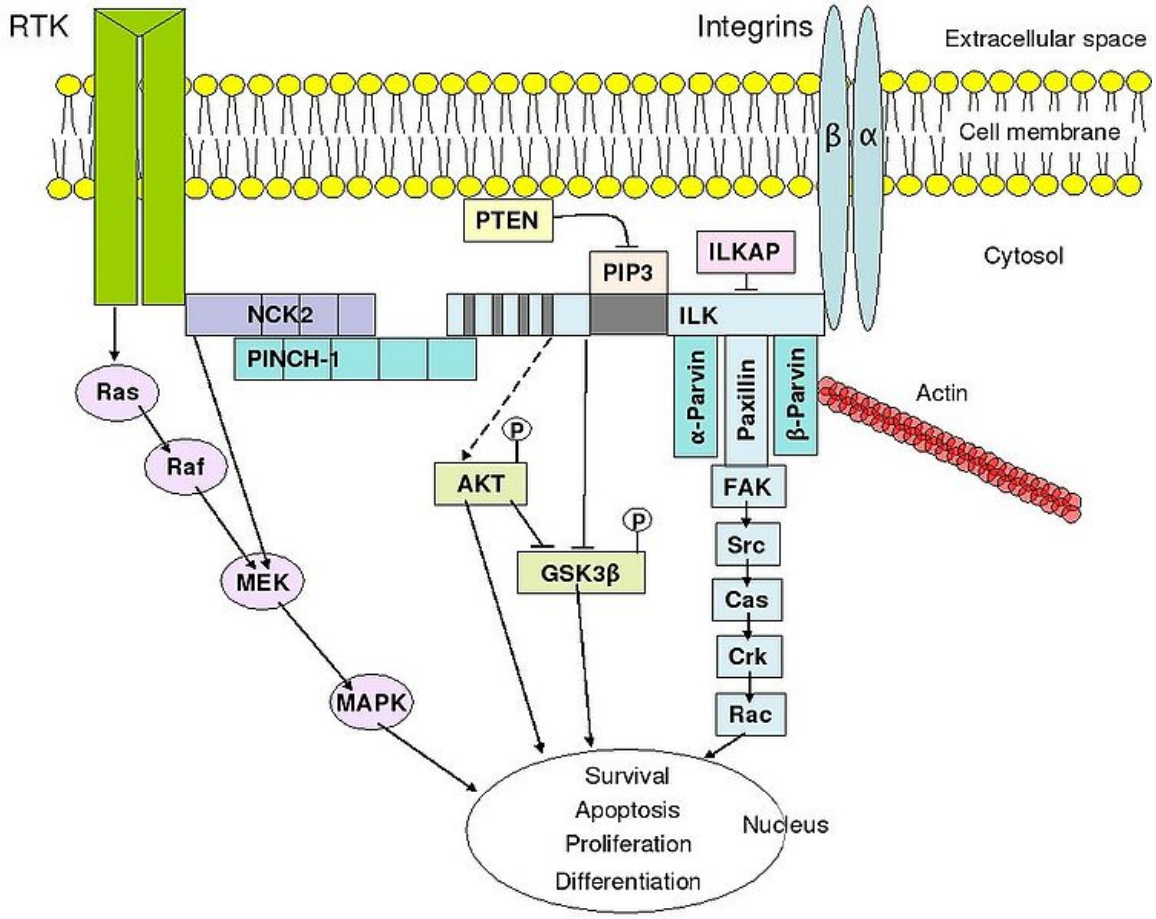
Receptor tyrosine kinases (RTKs) are transmembrane proteins with an intracellular kinase domain and an extracellular domain that binds ligand. There are many RTK proteins that are classified into subfamilies depending on their structural properties and ligand specificity. These include many growth factor receptors such as insulin receptor and the insulin-like growth factor receptors, and many others receptors. To conduct their biochemical signals, RTKs need to form dimers in the plasma membrane. The dimer is stabilized by ligand binding by the receptor. Interaction between the two cytoplasmic domains of the dimer is thought to stimulate autophosphorylation of tyrosines within the cytoplasmic tyrosine kinase domains of the RTKs causing their conformational changes. The kinase domain of the receptors is subsequently activated, initiating signaling cascades of phosphorylation of downstream cytoplasmic molecules. These signals are essential to various cellular processes, such as control of cell growth, differentiation, metabolism, and migration.

As is the case with G-Protein-coupled receptors, proteins that bind GTP play a major role in transmission of signal from the activated RTK into the cell. In this case, the G proteins are members of the Ras, Rho, and Raf families, referred to collectively as small G proteins. These proteins act as molecular switches that are usually tethered to membranes by isoprenyl groups linked to their carboxyl ends. Thus, upon activation, they are responsible for the recruitment of proteins to specific membrane subdomains where they participate in signaling. Activated RTKs, in turn, activate small G proteins, which in turn activate Guanine Nucleotide Exchange Factors, such as SOS1. Once activated, these

exchange factors can activate many more small G-proteins, thus amplifying the receptors initial signal.

As with the mutation of G-protein coupled receptors, the mutation of certain RTK genes can result in the expression of receptors that exist in a constitutively-activate state. Such mutated RTK genes may act as oncogenes, genes that contribute to the initiation or progression of cancer.

Integrins



An overview of integrin-mediated signal transduction, adapted from Hehlgens *et al.* (2007).

Integrins are produced by a wide variety of cell types, and play a role in the attachment of a cell to the extracellular matrix (ECM) and to other cells, and in the signal transduction of signals received from extracellular matrix components such as fibronectin, collagen, and laminin. Ligand-binding to the extracellular domain of integrins induces a conformational change within the protein and a clustering of the protein at the cell surface to initiate signal transduction. Integrins lack kinase activity, and integrin-mediated signal transduction is achieved through a variety of intracellular protein kinases

and adaptor molecules such as integrin-linked kinase (ILK), focal-adhesion kinase (FAK), talin, paxillin, parvins, p130Cas, Src-family kinases, and GTPases of the Rho family, the main protein coordinating signal transduction being ILK. As shown in the overview to the right, cooperative integrin and receptor tyrosine kinase signaling determine cellular survival, apoptosis, proliferation, and differentiation.

Important differences exist between integrin-signaling in circulating blood cells and that in non-circulating blood cells such as epithelial cells. Integrins at the cell-surface of circulating cells are inactive under normal physiological conditions. For example, cell-surface integrins on circulating leukocytes are maintained in an inactive state to avoid epithelial cell attachment. Only in response to appropriate stimuli are leukocyte integrins converted into an active form, such as those received at the site of an inflammatory response. In a similar manner, it is important that integrins at the cell surface of circulating platelets are kept in an inactive state under normal conditions to avoid thrombosis. Epithelial cells, in contrast, have active integrins at their cell surface under normal conditions, which help maintain their stable adhesion to underlying stromal cells, which provide appropriate signals to maintain their survival and differentiation.

Toll-like receptors

When activated, Toll-like receptors (TLRs) recruit adapter molecules within the cytoplasm of cells in order to propagate a signal. Four adapter molecules are known to be involved in signaling. These proteins are known as MyD88, Tirap (also called Mal), Trif, and Tram. The adapters activate other molecules within the cell, including certain protein kinases (IRAK1, IRAK4, TBK1, and IKKi) that amplify the signal, and ultimately lead to the induction or suppression of genes that orchestrate the inflammatory response. In all, thousands of genes are activated by TLR signaling, and, together, the TLRs constitute one of the most powerful and important gateways for gene modulation.

Ligand-gated ion channel receptors

A *ligand-activated ion channel* will recognize its ligand, and then undergo a structural change that opens a gap (channel) in the plasma membrane through which ions can pass. These ions will then relay the signal. An example for this mechanism is found in the receiving cell, or post-synaptic cell of a neural synapse.

By contrast, other ion channels open in response to a change in cell potential, that is, the difference of the electrical charge across the membrane. In neurons, this mechanism underlies the action potentials that travel along nerves. The influx of ions that occurs in response to ligand-gated ion channels often induce action potentials by depolarizing the membrane of the post-synaptic cells, which results in the wave-like opening of voltage-gated ion channels. In addition, calcium ions are also commonly allowed into the cell during ligand-induced ion channel opening. This calcium can act as a classical second messenger, setting in motion signal transduction cascades and altering the cellular physiology of the responding cell. This may result in strengthening of the synapse

between the pre- and post-synaptic cells by remodeling the dendritic spines involved in the synapse.

Intracellular receptors

Intracellular receptors include nuclear receptors and cytoplasmic receptors, and are soluble proteins localized within the nucleoplasm or the cytoplasm, respectively. The typical ligands for nuclear receptors are lipophilic hormones, with steroid hormones (for example, testosterone, progesterone, and cortisol) and derivatives of vitamin A and D among them. To reach its receptor and initiate signal transduction, the hormone must pass through the plasma membrane, usually by passive diffusion. The nuclear receptors are ligand-activated transcription activators; on binding with the ligand (the hormone), the ligands will pass through the nuclear membrane into the nucleus and enable the transcription of a certain gene and, thus, the production of a protein.

The nuclear receptors that were activated by the hormones attach at the DNA at receptor-specific *Hormone-Responsive Elements* (*HREs*), DNA sequences that are located in the promoter region of the genes that are activated by the hormone-receptor complex. As this enables the transcription of the according gene, these hormones are also called *inductors of gene expression*. The activation of gene transcription is much slower than signals that directly affect existing proteins. As a consequence, the effects of hormones that use nucleic receptors are usually long-term. Although the signal transduction via these soluble receptors involves only a few proteins, the details of gene regulation are yet not well understood. The nucleic receptors all have a similar, modular structure:

N-AAAABBBBCCCCDDDDEEEEFFFF-C

where CCCC is the DNA-binding domain that contains zinc fingers, and EEEE the ligand-binding domain. The latter is also responsible for dimerization of most nuclearic receptors prior to DNA binding. As a third function, it contains structural elements that are responsible for transactivation, used for communication with the translational apparatus. The zinc fingers in the DNA-binding domain stabilize DNA binding by holding contact to the phosphate backbone of the DNA. The DNA sequences that match the receptor are usually hexameric repeats, either normal, inverted, or everted. The sequences are quite similar, but their orientation and distance are the parameters by which the DNA-binding domains of the receptors can tell them apart.

Steroid receptors are a subclass of nuclear receptors, located primarily within the cytosol. In the absence of steroid hormone, the receptors cling together in a complex called an *aporeceptor complex*, which also contains chaperone proteins (also known as *heatshock proteins* or *Hsp*s). The *Hsp*s are necessary to activate the receptor by assisting the protein to fold in a way such that the signal sequence that enables its passage into the nucleus is accessible.

Steroid receptors can also have a *repressive* effect on gene expression, when their transactivation domain is hidden so it cannot activate transcription. Furthermore, steroid

receptor activity can be enhanced by phosphorylation of serine residues at their N-terminal end, as a result of another signal transduction pathway, for example, a by a growth factor. This behaviour is called *crosstalk*.

RXR- and orphan-receptors These nuclear receptors can be activated by

- a *classic* endocrine-synthesized hormone that entered the cell by diffusion
- a hormone that was built within the cell (for example, retinol) from a precursor or prohormone, which can be brought to the cell through the bloodstream
- a hormone that was completely synthesized within the cell, for example, prostaglandin.

These receptors are located in the nucleus and are *not* accompanied by *chaperone proteins*. In the absence of hormone, they bind to their specific DNA sequence, repressing the gene. Upon activation by the hormone, they activate the transcription of the gene that they were repressing.

Certain intracellular receptors of the immune system are examples of cytoplasmic receptors. Recently-identified NOD like receptors (NLRs) reside in the cytoplasm of specific eukaryotic cells and interact with particular ligands, such as microbial molecules, using a leucine-rich repeat (LRR) motif that is similar to the ligand-binding motif of the extracellular receptors known as TLRs. Some of these molecules (e.g., NOD1 and NOD2) interact with an enzyme called RICK kinase (or RIP2 kinase) that activates NF-κB signaling, whereas others (e.g., NALP3) interact with inflammatory caspases (e.g., caspase 1) and initiate processing of particular cytokines (e.g., interleukin-1β). Similar receptors exist inside plant cells and are called Plant R Proteins. Another type of cytoplasmic receptor also has a role in immune surveillance. These receptors are known as RNA Helicases and include RIG-I, MDA5, and LGP2.

Second messengers

Intracellular signal transduction is largely carried out by second messenger molecules.

Calcium

Ca^{2+} concentration is usually maintained at a very low level in the cytosol by sequestration in the smooth endoplasmic reticulum and the mitochondria. Ca^{2+} release from the endoplasmic reticulum into the cytosol results in the binding of the released Ca^{2+} to signaling proteins that are then activated. There are two combined receptor/ion channel proteins that perform the task of controlled transport of Ca^{2+}:

- The *$InsP_3$-receptor* will transport Ca^{2+} upon interaction with inositol triphosphate (thus the name) on its cytosolic side. It consists of four identical subunits.
- The *ryanodine receptor* is named after the plant alkaloid ryanodine. It is similar to the $InsP_3$ receptor and stimulated to transport Ca^{2+} into the cytosol by *recognizing Ca^{2+} on its cytosolic side*, thus establishing a feedback mechanism; a

small amount of Ca^{2+} in the cytosol near the receptor will cause it to release even more Ca^{2+}. It is especially important in neurons and muscle cells. In heart and pancreas cells, another second messenger (cyclic-ADP ribose) takes part in the receptor activation. The localized and time-limited activity of Ca^{2+} in the cytosol is also called a *Ca^{2+} wave*. Once released into the cytosol from intracellular stores or extracellular sources, Ca^{2+} acts as a signal molecule within the cell. This works by tightly limiting the time and space when Ca^{2+} is free (and thus active). Therefore, the concentration of free Ca^{2+} within the cell is usually very low; it is stored within organelles, usually the endoplasmic reticulum (sarcoplasmic reticulum in muscle cells), where it is bound to molecules like calreticulin.

Ca^{2+} is used in a multitude of processes, among them muscle contraction, release of neurotransmitter from nerve endings, vision in retina cells, proliferation, secretion, cytoskeleton management, cell migration, gene expression, and metabolism. The three main pathways that lead to Ca^{2+} activation are :

1. G protein-regulated pathways
2. Pathways regulated by receptor-tyrosine kinases
3. Ligand- or current-regulated ion channels

There are two different ways by which Ca^{2+} can regulate proteins:

1. A direct recognition of Ca^{2+} by the protein
2. Binding of Ca^{2+} in the active site of an enzyme.

One of the best-studied interactions of Ca^{2+} with a protein is the regulation of calmodulin by Ca^{2+}. Calmodulin itself can regulate other proteins, or be part of a larger protein (for example, phosphorylase kinase). The Ca^{2+}/calmodulin complex plays an important role in proliferation, mitosis, and neural signal transduction.

Lipophilic

Lipophilic second messenger molecules are derived from lipids that normally reside in cellular membranes. Enzymes stimulated by activated receptors modify the lipids, converting them into second messengers.

Diacylglycerol is a lipophilic second messenger, required for the activation of protein kinase C. Ceramide, the eicosanoids, and lysophosphatidic acid are also lipophilic second messengers.

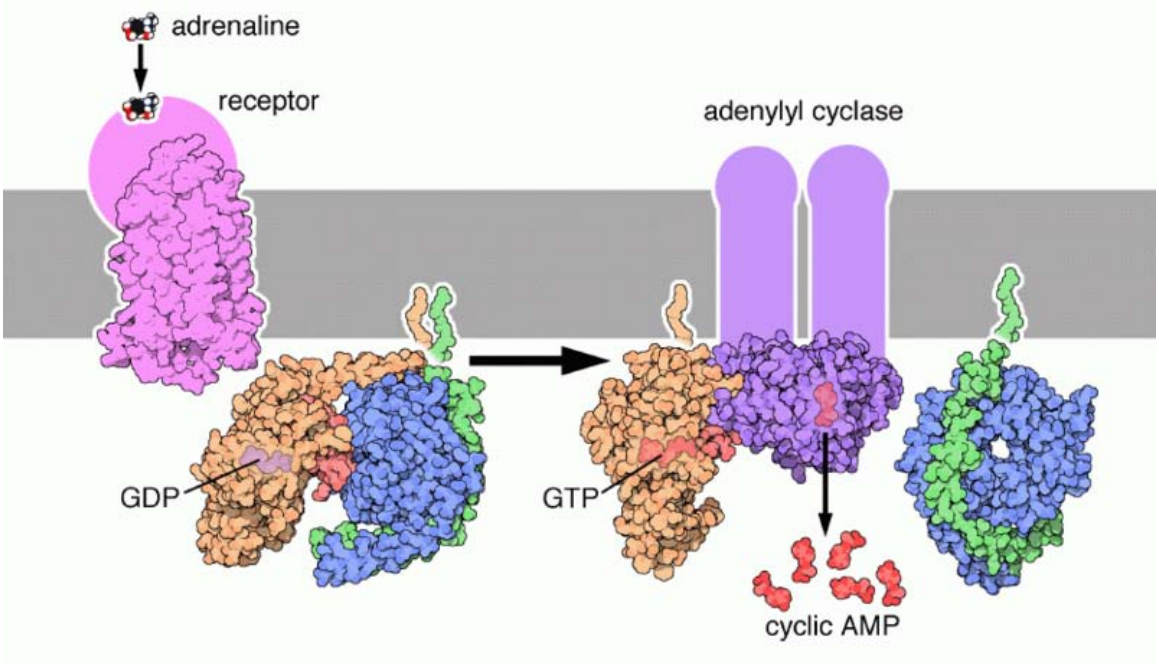
Nitric oxide

Nitric oxide (NO) can act as a second messenger. Nitric oxide gas is a free radical that freely diffuses through the plasma membrane and affects nearby cells. NO is made from arginine and oxygen by the enzyme NO synthase, with citrulline as a by-product. NO works mainly through activation of its target receptor, the enzyme soluble guanylate

cyclase, which, when activated, produces the second messenger cyclic-guanosine monophosphate (cGMP). NO can also act through covalent modification of proteins or their metal co-factors. Some of these modifications are reversible and work through a redox mechanism. NO is toxic in high concentrations, and is thought to cause damage during stroke.

NO is involved in a number of functions, including relaxation of blood vessels; regulation of exocytosis of neurotransmitters; cellular immune response; modulation of the Hair Cycle; production and maintenance of penile erections; and activation of apoptosis by initiating signals that lead to H2AX phosphorylation.

Major pathway examples



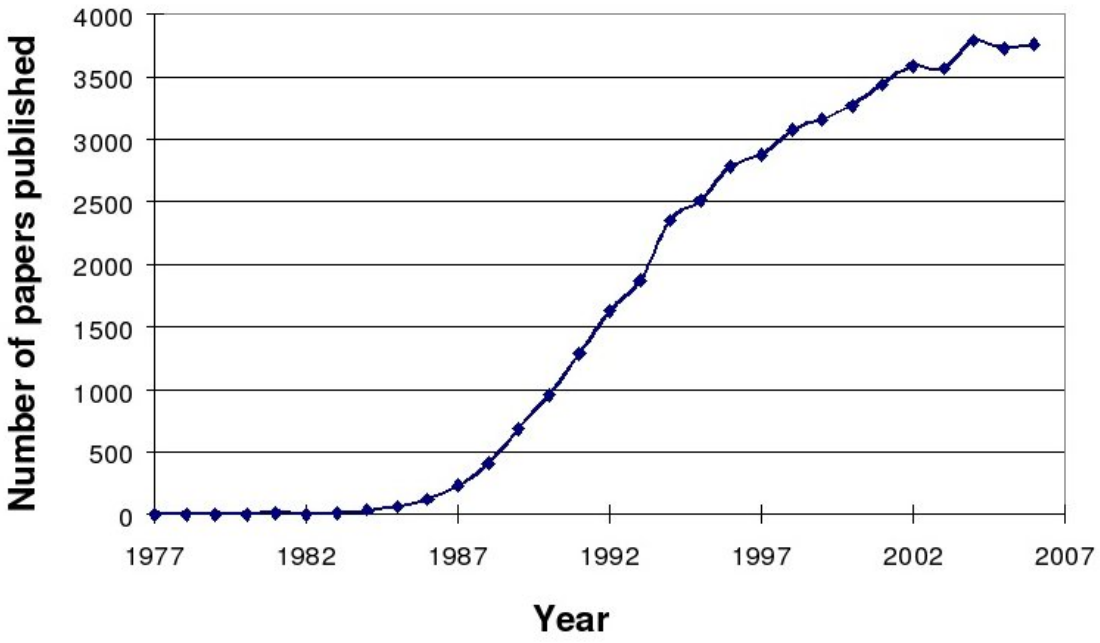
Mechanism of cAMP dependent signaling. In this figure, the neurotransmitter epinephrine (adrenaline) and its receptor (pink) is used as an example. The activated receptor releases the G_s alpha protein (tan) from the beta amd gamma subunits (blue and green) in the heterotrimeric G-protein complex. The activated G_s alpha protein in turn activates adenylyl cyclase (purple) that converts ATP into the second messenger cAMP.

- cAMP dependent pathway: In humans, cAMP works by activating protein kinase A (PKA, cAMP-dependent protein kinase), and thus, further effects mainly depend on cAMP-dependent protein kinase, which vary based on the type of cell.

- MAPK/ERK pathway: A pathway that couples intracellular responses to the binding of growth factors to cell surface receptors. This pathway is very complex and includes many protein components. The basic pathway shown in the figure (to the right) and described below includes the major components of the pathway. In many cell types, activation of this pathway promotes cell division.

- IP_3/DAG pathway: PLC cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) yielding diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG remains bound to the membrane, and IP_3 is released as a soluble structure into the cytosol. IP_3 then diffuses through the cytosol to bind to IP_3 receptors, particular calcium channels in the endoplasmic reticulum (ER). These channels are specific to calcium and only allow the passage of calcium to move through. This causes the cytosolic concentration of Calcium to increase, causing a cascade of intracellular changes and activity. In addition, calcium and DAG together works to activate PKC, which goes on to phosphorylate other molecules, leading to altered cellular activity. End effects include taste, manic depression, tumor promotion, etc.

History



Occurrence of the term "signal transduction" The total number of papers published in each year since 1977 containing the phrase *signal transduction* in either their title or abstract section are plotted. These figures were extracted through an analysis of the papers contained within the MEDLINE database.

The earliest scientific paper recorded in the MEDLINE database as containing the specific term *signal transduction* within its text was published in 1972.

Some articles published before 1977 use the term *signal transmission* or *sensory transduction* in their titles or abstracts but it was not until 1977 that papers began to be published with the specific term *signal transduction* in their abstracts, and it was not until 1979 that the term appeared within a paper title.. One source attributes the widespread use of the term *signal transduction* to a 1980 review article by Rodbell. As can be seen

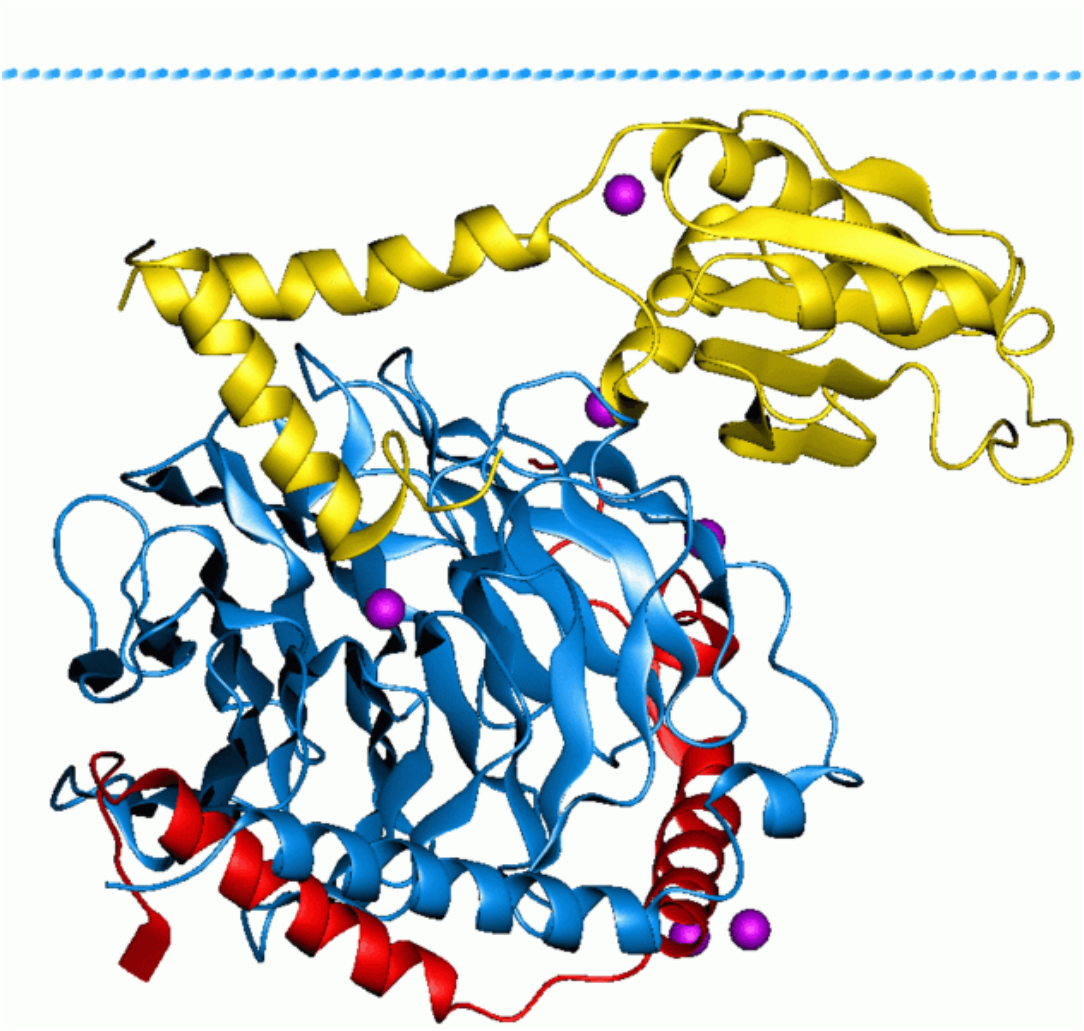
from the graph to the right, research papers directly addressing signal transduction processes began to appear in large numbers in the scientific literature in the late 1980s and early 1990s.

One notable early discovery in the field of signal transduction was the link Rodbell made between metabolic regulation and the activity of GTP and GTP-binding proteins. The current understanding of signal transduction processes reflects contributions made over many years by research groups all over the world.

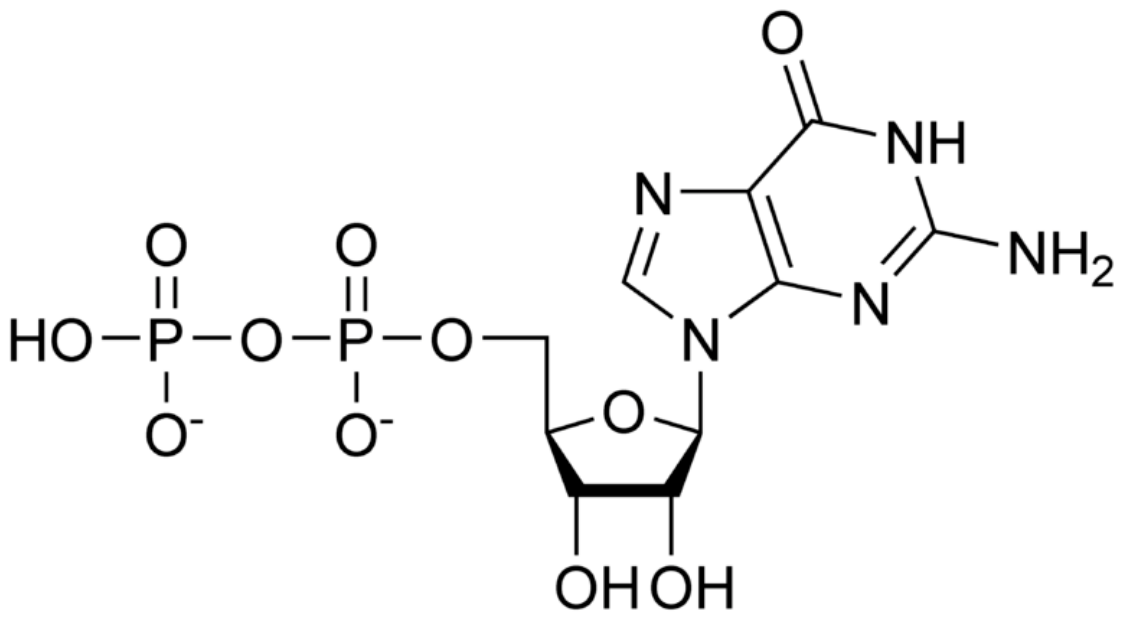
A total of 48,377 scientific papers related to signal transduction were published in 1977; of these, 11,211 were reviews of other papers.

Chapter- 7

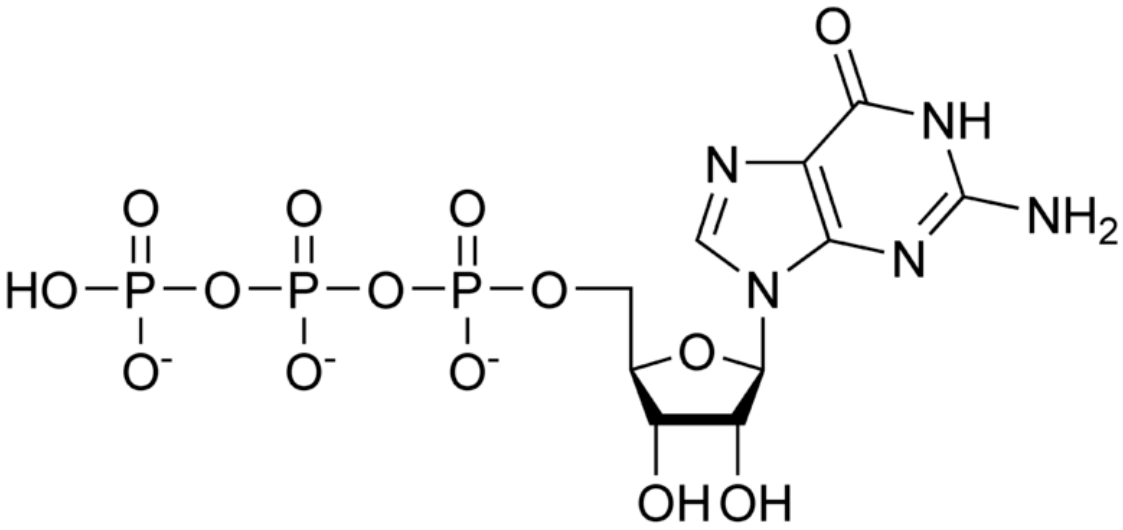
G protein



Phosducin- transducin beta-gamma complex. Beta and gamma subunits of G-protein are shown by blue and red, respectively.



Guanosine diphosphate



Guanosine triphosphate

G proteins (**guanine nucleotide-binding proteins**) are a family of proteins involved in transmitting chemical signals outside the cell, and causing changes inside the cell. They communicate signals from many hormones, neurotransmitters, and other signaling factors.

G protein-coupled receptors are transmembrane receptors. Signal molecules bind to a domain located outside the cell. An intracellular domain activates a G protein. The G

protein activates a cascade of further compounds, and finally causes a change downstream in the cell.

G protein complexes bind to phosphate groups. They function as molecular switches. When they are attached to a complex with three phosphate groups (guanosine triphosphate [GTP]), they turn on. When they are attached to a complex with only two phosphate groups (guanosine diphosphate [GDP]), they turn off.

G proteins regulate metabolic enzymes, ion channels, transporters, and other parts of the cell machinery, controlling transcription, motility, contractility, and secretion, which in turn regulate systemic functions such as embryonic development, learning and memory, and homeostasis.

G proteins were discovered when Alfred G. Gilman and Martin Rodbell investigated stimulation of cells by adrenaline. They found that, when adrenaline binds to a receptor, the receptor does not stimulate enzymes directly. Instead, the receptor stimulates a G protein, which stimulates an enzyme. An example is adenylate cyclase, which produces the second messenger cyclic AMP. For this discovery, they won the 1994 Nobel Prize in Physiology or Medicine.

G proteins belong to the larger group of enzymes called GTPases.

Function

G proteins are important signal transducing molecules in cells. "Malfunction of GPCR [G Protein-Coupled Receptor] signaling pathways are involved in many diseases, such as diabetes, blindness, allergies, depression, cardiovascular defects, and certain forms of cancer. It is estimated that more than half of the modern drugs' cellular targets are GPCRs."

The human genome encodes roughly 950 G protein-coupled receptors, which detect photons (light), hormones, growth factors, drugs, and other endogenous ligands. Approximately 150 of the GPCRs found in the human genome have unknown functions.

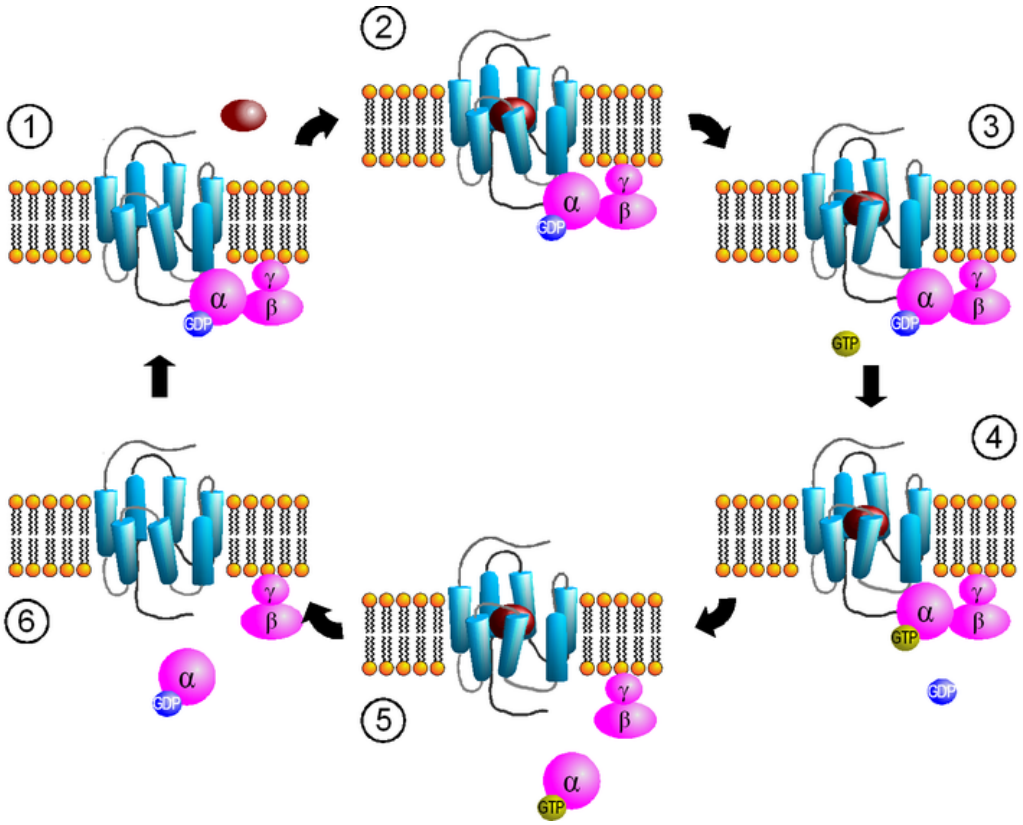
Types of G protein signaling

G protein can refer to two distinct families of proteins. Heterotrimeric G proteins, sometimes referred to as the "large" G proteins that are activated by G protein-coupled receptors and made up of alpha (α), beta (β), and gamma (γ) subunits. There are also *"small" G proteins* (20-25kDa) that belong to the Ras superfamily of small GTPases. These proteins are homologous to the alpha (α) subunit found in heterotrimers, and are in fact monomeric. However, they also bind GTP and GDP and are involved in signal transduction.

Heterotrimeric G proteins

Different types of heterotrimeric G proteins share a common mechanism. They are activated in response to a conformation change in the G protein-coupled receptor, exchange GDP for GTP, and dissociate to activate other proteins in the signal transduction pathway. The specific mechanisms, however, differ among the types.

Common mechanism



Activation cycle of G-proteins by G-protein-coupled receptors

Receptor-activated G proteins are bound to the inside surface of the cell membrane. They consist of the G_α and the tightly associated $G_{\beta\gamma}$ subunits. There are many classes of G_α subunits: $G_s\alpha$ (G stimulatory), $G_i\alpha$ (G inhibitory), $G_o\alpha$ (G other), $G_{q/11}\alpha$, and $G_{12/13}\alpha$ are some examples. They behave differently in the recognition of the effector, but share a similar mechanism of activation.

Activation

When a ligand activates the G protein-coupled receptor, it induces a conformational change in the receptor that allows the receptor to function as a guanine nucleotide exchange factor (GEF) that exchanges GDP for GTP on the G_α subunit. In the traditional view of heterotrimeric protein activation, this exchange triggers the dissociation of the G_α

subunit, bound to GTP, from the $G_{\beta\gamma}$ dimer and the receptor. However, models that suggest molecular rearrangement, reorganization, and pre-complexing of effector molecules are beginning to be accepted. Both G_{α}-GTP and $G_{\beta\gamma}$ can then activate different *signaling cascades* (or *second messenger pathways*) and effector proteins, while the receptor is able to activate the next G protein.

Termination

The G_{α} subunit will eventually hydrolyze the attached GTP to GDP by its inherent enzymatic activity, allowing it to re-associate with $G_{\beta\gamma}$ and starting a new cycle. A group of proteins called Regulator of G protein signalling (RGSs), act as GTPase-activating proteins (GAPs), specific for G_{α} subunits. These proteins act to accelerate hydrolysis of GTP to GDP and terminate the transduced signal. In some cases, the effector itself may possess intrinsic GAP activity, which helps deactivate the pathway. This is true in the case of phospholipase C beta, which possesses GAP activity within its C-terminal region. This is an alternate form of regulation for the G_{α} subunit.

Specific mechanisms

- $\mathbf{G_{\alpha s}}$ activates the cAMP-dependent pathway by stimulating the production of cAMP from ATP. This is accomplished by direct stimulation of the membrane-associated enzyme adenylate cyclase. cAMP acts as a second messenger that goes on to interact with and activate protein kinase A (PKA). PKA can then phosphorylate myriad downstream targets.
- $\mathbf{G_{\alpha i}}$ inhibits the production of cAMP from ATP.
- $\mathbf{G_{\alpha q/11}}$ stimulates membrane-bound phospholipase C beta, which then cleaves PIP_2 (a minor membrane phosphoinositol) into two second messengers, IP3 and diacylglycerol (DAG).
- $\mathbf{G_{\alpha 12/13}}$ are involved in Rho family GTPase signaling (through RhoGEF superfamily) and control cell cytoskeleton remodeling, thus regulating cell migration.
- $\mathbf{G_{\beta\gamma}}$ sometimes also have active functions, e.g., coupling to and activating GIRK Channels.

Small GTPases

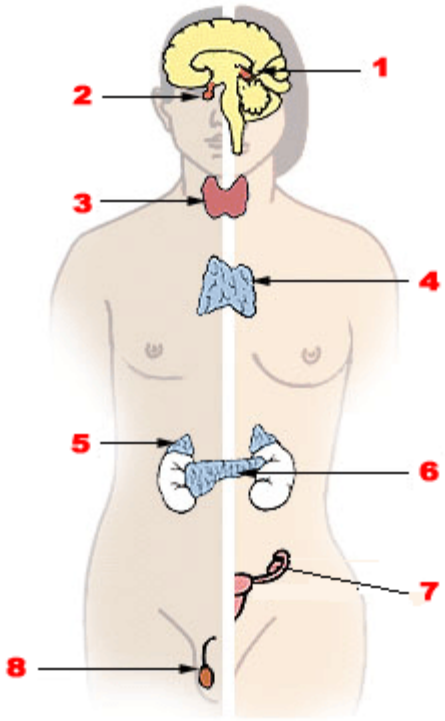
Small GTPases also bind GTP and GDP and are involved in signal transduction. These proteins are homologous to the alpha (α) subunit found in heterotrimers, but exist as monomers. They are small (20-kDa to 25-kDa) proteins that bind to guanosine triphosphate (GTP). This family of proteins is homologous to Ras GTPases and is also called the Ras superfamily GTPases.

Lipidation

In order to associate with the inner leaflet of the plasma membrane, many G proteins and small GTPases are lipidated, that is, covalently modified with lipid extensions. They may be myristolated, palmitoylated or prenylated.

Chapter- 8

Endocrine System



Major endocrine glands. (Male on the left, female on the right.) ***1.*** *Pineal gland* ***2.*** *Pituitary gland* ***3.*** *Thyroid gland* ***5.*** *Adrenal gland* ***6.*** *Pancreas* ***7.*** *Ovary* ***8.*** *Testis.* [Note: the Thymus (labelled 4.) has endocrine function, though it is not considered to be an endocrine gland.]

In physiology, the **endocrine system** is a system of glands, each of which secretes a type of hormone into the bloodstream to regulate the body. It derives from the Greek words endo (Greek *ένδο*) meaning inside, within, and crinis (Greek *κρινής*) for secrete. The endocrine system is an information signal system like the nervous system. Hormones are substances (chemical mediators) released from endocrine tissue into the

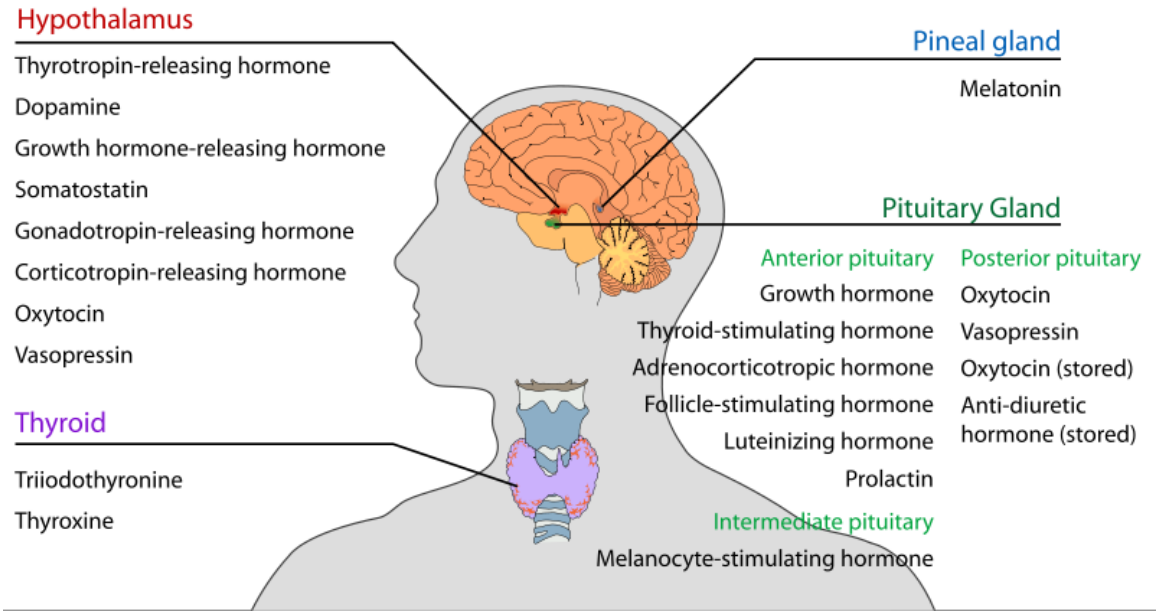
bloodstream that attach to target tissue and allow communication among cells. Hormones regulate many functions of an organism, including mood, growth and development, tissue function, and metabolism. The field of study that deals with disorders of endocrine glands is endocrinology, a branch of internal medicine.

The endocrine system is made up of a series of ductless glands that produce chemicals called hormones. A number of glands that signal each other in sequence is usually referred to as an axis, for example, the hypothalamic-pituitary-adrenal axis. Typical endocrine glands are the pituitary, thyroid, and adrenal glands. Features of endocrine glands are, in general, their ductless nature, their vascularity, and usually the presence of intracellular vacuoles or granules storing their hormones. In contrast, exocrine glands, such as salivary glands, sweat glands, and glands within the gastrointestinal tract, tend to be much less vascular and have ducts or a hollow lumen.

In addition to the specialised endocrine organs mentioned above, many other organs that are part of other body systems, such as the kidney, liver, heart and gonads, have secondary endocrine functions. For example the kidney secretes endocrine hormones such as erythropoietin and renin.

Endocrine organs and secreted hormones

Central nervous system



Endocrine glands in the human head and neck and their hormones

Hypothalamus

Secreted hormone	Abbreviation	Produced by	Effect
Thyrotropin-releasing hormone (Prolactin-releasing hormone)	TRH, TRF, or PRH	Parvocellular neurosecretory neurons	Stimulate thyroid-stimulating hormone (TSH) released from anterior pituitary (primarily) Stimulate prolactin release from anterior pituitary
Dopamine (Prolactin-inhibiting hormone)	DA or PIH	Dopamine neurons of the arcuate nucleus	Inhibit prolactin released from anterior pituitary
Growth hormone-releasing hormone	GHRH	Neuroendocrine neurons of the Arcuate nucleus	Stimulate Growth hormone (GH) release from anterior pituitary
Somatostatin (growth hormone-inhibiting hormone)	SS, GHIH, or SRIF	Neuroendocrine cells of the Periventricular nucleus	Inhibit Growth hormone (GH) release from anterior pituitary Inhibit thyroid-stimulating hormone (TSH) release from anterior pituitary
Gonadotropin-releasing hormone	GnRH or LHRH	Neuroendocrine cells of the Preoptic area	Stimulate follicle-stimulating hormone (FSH) release from anterior pituitary Stimulate luteinizing hormone (LH) release from anterior pituitary
Corticotropin-releasing hormone	CRH or CRF	Parvocellular neurosecretory neurons or the Paraventricular Nucleus	Stimulate adrenocorticotropic hormone (ACTH) release from anterior pituitary
Oxytocin	OT or OXT	Magnocellular neurosecretory neurons of the Supraoptic Nucleus and Paraventricular Nucleus	Uterine contraction Lactation (letdown reflex)
Vasopressin (antidiuretic hormone)	ADH or AVP or VP	Parvocellular neurosecretory neurons, Magnocellular neurosecretory neurons of the	Increases water permeability in the distal convoluted tubule and collecting duct of nephrons, thus promoting water reabsorption and increasing blood volume

	Paraventricular Nucleus and Supraoptic Nucleus	

Pineal body (epiphysis)

Secreted hormone	From cells	Effect
Melatonin	Pinealocytes	Antioxidant Monitors the circadian rhythm including inducement of drowsiness and lowering of the core body temperature

Pituitary Gland (hypophysis)

Anterior pituitary lobe (adenohypophysis)

Secreted hormone	Abbreviation	From cells	Effect
Growth hormone (somatotropin)	GH	Somatotrophs	Stimulates growth and cell reproduction Stimulates Insulin-like growth factor 1 release from liver
Thyroid-stimulating hormone (thyrotropin)	TSH	Thyrotrophs	Stimulates thyroxine (T4) and triiodothyronine (T3) synthesis and release from thyroid gland Stimulates iodine absorption by thyroid gland
Adrenocorticotropic hormone (corticotropin)	ACTH	Corticotrophs	Stimulates corticosteroid (glucocorticoid and mineralcorticoid) and androgen synthesis and release from adrenocortical cells
Beta-endorphin	-	Corticotrophs	Inhibits perception of pain
Follicle-stimulating hormone	FSH	Gonadotrophs	In females: Stimulates maturation of ovarian follicles in ovary In males: Stimulates maturation of seminiferous tubules In males: Stimulates spermatogenesis In males: Stimulates production of androgen-binding protein from Sertoli cells of the testes
Luteinizing hormone	LH	Gonadotrophs	In females: Stimulates ovulation In females: Stimulates formation of corpus luteum

			In males: Stimulates testosterone synthesis from Leydig cells (interstitial cells)
Prolactin	PRL	Lactotrophs	Stimulates milk synthesis and release from mammary glands Mediates sexual gratification

Posterior pituitary lobe (neurohypophysis)

Secreted hormone	Abbreviation	From cells	Effect
Oxytocin		Magnocellular neurosecretory cells	Uterine contraction Lactation (letdown reflex)
Vasopressin (antidiuretic hormone)	ADH or AVP	Parvocellular neurosecretory neurons	Increases water permeability in the distal convoluted tubule and collecting duct of nephrons, thus promoting water reabsorption and increasing blood volume

Oxytocin and anti-diuretic hormone are not secreted in the posterior lobe, merely stored.

Intermediate pituitary lobe (pars intermedia)

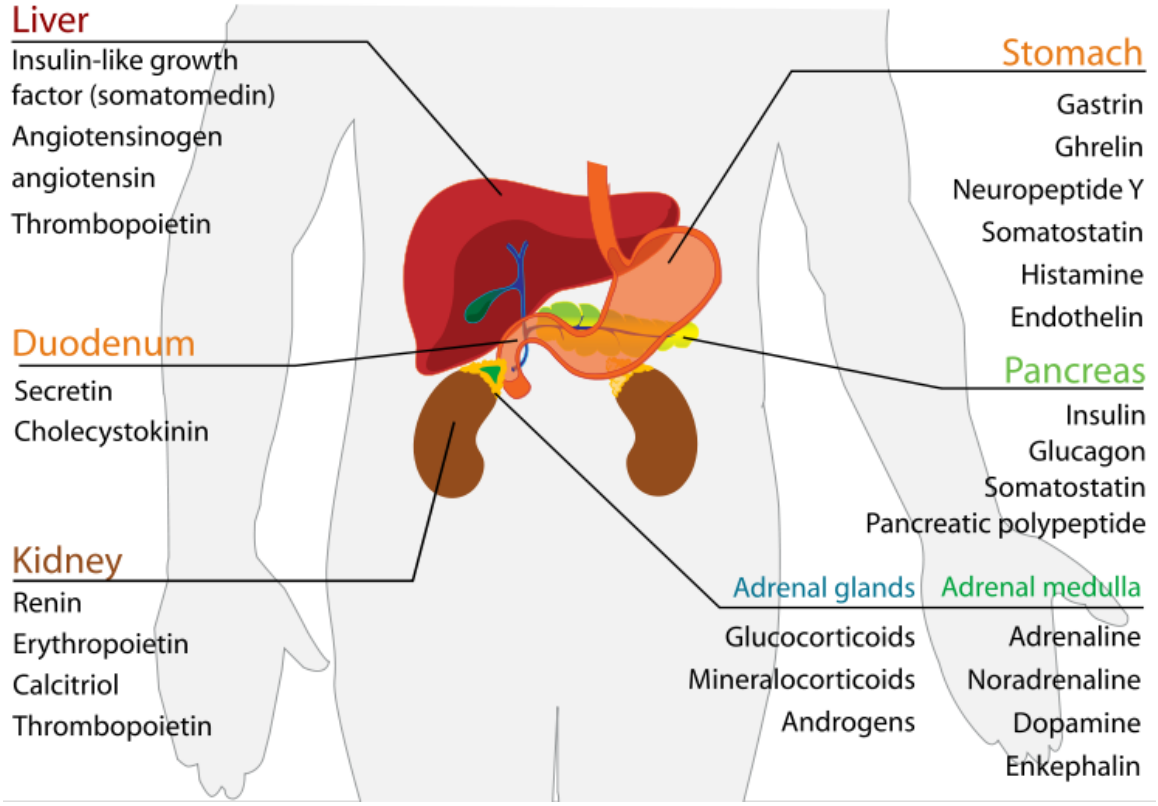
Secreted hormone	Abbreviation	From cells	Effect
Melanocyte-stimulating hormone	MSH	Melanotropes	Stimulates melanin synthesis and release from skin/hair melanocytes

Thyroid

Secreted hormone	Abbreviation	From cells	Effect
Triiodothyronine	T3	Thyroid epithelial cell	(More potent form of thyroid hormone) Stimulates body oxygen and energy consumption, thereby increasing the basal metabolic rate Stimulates RNA polymerase I and II, thereby promoting protein synthesis
Thyroxine (tetraiodothyronine)	T4	Thyroid epithelial cells	(Less active form of thyroid hormone) (Acts as a prohormone to triiodothyronine) Stimulates body oxygen and energy consumption, thereby increasing the basal metabolic rate

		Stimulates RNA polymerase I and II, thereby promoting protein synthesis
Calcitonin	Parafollicular cells	Stimulates osteoblasts and thus bone construction Inhibits Ca^{2+} release from bone, thereby reducing blood Ca^{2+}

Alimentary system



Stomach

Secreted hormone	Abbreviation	From cells	Effect
Gastrin (Primarily)		G cells	Secretion of gastric acid by parietal cells
Ghrelin		P/D1 cells	Stimulate appetite, secretion of growth hormone from anterior pituitary gland
Neuropeptide Y	NPY		increased food intake and decreased physical activity
Somatostatin		D cells	Suppress release of gastrin,

		cholecystokinin (CCK), secretin, motilin, vasoactive intestinal peptide (VIP), gastric inhibitory polypeptide (GIP), enteroglucagon Lowers rate of gastric emptying Reduces smooth muscle contractions and blood flow within the intestine.
Histamine	ECL cells	stimulate gastric acid secretion
Endothelin	X cells	Smooth muscle contraction of stomach

Duodenum

Secreted hormone	From cells	Effect
Secretin	S cells	Secretion of bicarbonate from liver, pancreas and duodenal Brunner's glands Enhances effects of cholecystokinin Stops production of gastric juice
Cholecystokinin	I cells	Release of digestive enzymes from pancreas Release of bile from gallbladder hunger suppressant

Liver

Secreted hormone	Abbreviation	From cells	Effect
Insulin-like growth factor (or somatomedin) (Primarily)	IGF	Hepatocytes	insulin-like effects regulate cell growth and development
Angiotensinogen and angiotensin		Hepatocytes	vasoconstriction release of aldosterone from adrenal cortex dipsogen.
Thrombopoietin		Hepatocytes	stimulates megakaryocytes to produce platelets

Pancreas

Secreted hormone	From cells	Effect
Insulin (Primarily)	β Islet cells	Intake of glucose, glycogenesis and glycolysis in liver and muscle from blood intake of lipids and synthesis of triglycerides in adipocytes Other anabolic effects
Glucagon (Also Primarily)	α Islet cells	glycogenolysis and gluconeogenesis in liver increases blood glucose level
Somatostatin	δ Islet cells	Inhibit release of insulin Inhibit release of glucagon Suppress the exocrine secretory action of pancreas.
Pancreatic polypeptide	PP cells	Self regulate the pancreas secretion activities and effect the hepatic glycogen levels.

Kidney

Secreted hormone	From cells	Effect
Renin (Primarily)	Juxtaglomerular cells	Activates the renin-angiotensin system by producing angiotensin I of angiotensinogen
Erythropoietin (EPO)	Extraglomerular mesangial cells	Stimulate erythrocyte production
Calcitriol (1,25-dihydroxyvitamin D_3)		Active form of vitamin D_3 Increase absorption of calcium and phosphate from gastrointestinal tract and kidneys inhibit release of PTH
Thrombopoietin		stimulates megakaryocytes to produce platelets

Adrenal glands

Adrenal cortex

Secreted hormone	From cells	Effect
Glucocorticoids (chiefly cortisol)	zona fasciculata and zona reticularis cells	Stimulates gluconeogenesis Stimulates fat breakdown in adipose tissue Inhibits protein synthesis Inhibits glucose uptake in muscle and

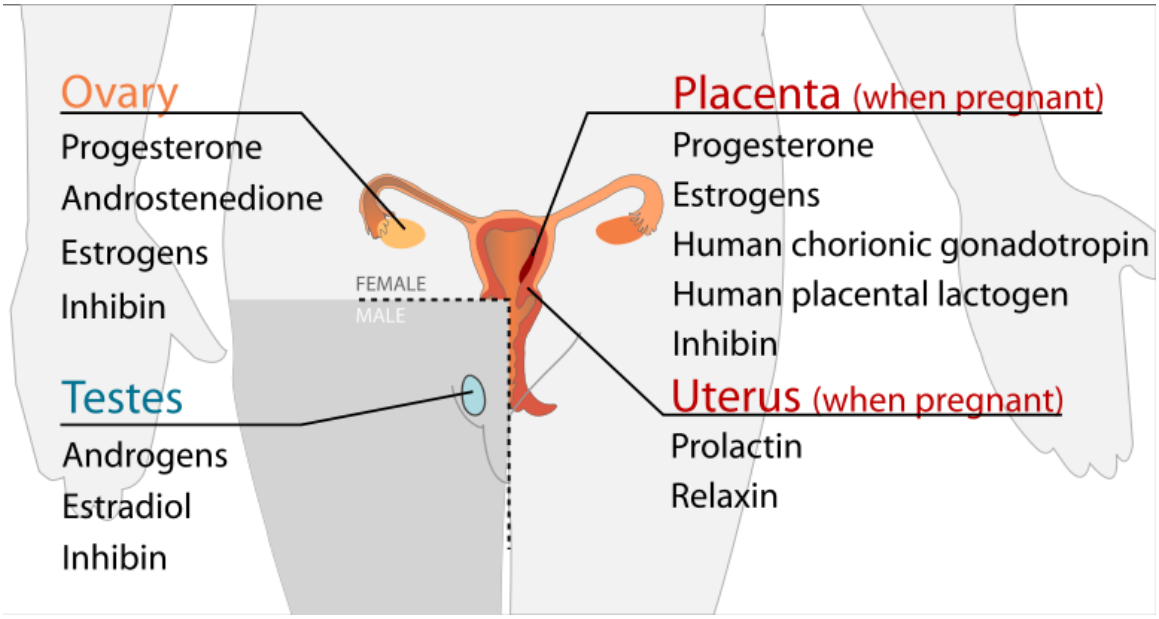
		adipose tissue Inhibits immunological responses (immunosuppressive) Inhibits inflammatory responses (anti-inflammatory)
Mineralocorticoids (chiefly aldosterone)	Zona glomerulosa cells	Stimulates active sodium reabsorption in kidneys Stimulates passive water reabsorption in kidneys, thus increasing blood volume and blood pressure Stimulates potassium and H^+ secretion into nephron of kidney and subsequent excretion
Androgens (including DHEA and testosterone)	Zona fasciculata and Zona reticularis cells	In males: Relatively small effect compared to androgens from testes In females: masculinizing effects (ie. excessive facial hair)

Adrenal medulla

Secreted hormone	From cells	Effect
Adrenaline (epinephrine) (Primarily)	Chromaffin cells	Fight-or-flight response: • Boost the supply of oxygen and glucose to the brain and muscles (by increasing heart rate and stroke volume, vasodilation, increasing catalysis of glycogen in liver, breakdown of lipids in fat cells) • Dilate the pupils • Suppress non-emergency bodily processes (e.g., digestion) • Suppress immune system
Noradrenaline (norepinephrine)	Chromaffin cells	Fight-or-flight response: • Boost the supply of oxygen and glucose to the brain and muscles (by increasing heart rate and stroke volume, vasoconstriction and increased blood pressure, breakdown of lipids in fat cells) • Increase skeletal muscle readiness.
Dopamine	Chromaffin cells	Increase heart rate and blood pressure

Enkephalin	Chromaffin cells	Regulate pain

Reproductive



Testes

Secreted hormone	From cells	Effect
Androgens (chiefly testosterone)	Leydig cells	Anabolic: growth of muscle mass and strength, increased bone density, growth and strength, Virilizing: maturation of sex organs, formation of scrotum, deepening of voice, growth of beard and axillary hair.
Estradiol	Sertoli cells	Prevent apoptosis of germ cells
Inhibin	Sertoli cells	Inhibit production of FSH

Ovarian follicle / Corpus luteum

Secreted hormone	From cells	Effect
Progesterone	Granulosa cells, theca cells	Support pregnancy: • Convert endometrium to secretory stage • Make cervical mucus permeable to sperm. • Inhibit immune response, e.g.,

		towards the human embryo • Decrease uterine smooth muscle contractility • Inhibit lactation • Inhibit onset of labor. Other: • Raise epidermal growth factor-1 levels • Increase core temperature during ovulation • Reduce spasm and relax smooth muscle (widen bronchi and regulate mucus) Anti-inflammatory • Reduce gall-bladder activity • Normalize blood clotting and vascular tone, zinc and copper levels, cell oxygen levels, and use of fat stores for energy • Assist in thyroid function and bone growth by osteoblasts • Increase resilience in bone, teeth, gums, joint, tendon, ligament, and skin • Promote healing by regulating collagen • Provide nerve function and healing by regulating myelin • Prevent endometrial cancer by regulating effects of estrogen
Androstenedione	Theca cells	Substrate for estrogen
Estrogens (mainly estradiol)	Granulosa cells	Structural: • Promote formation of female secondary sex characteristics • Accelerate height growth • Accelerate metabolism (burn fat) • Reduce muscle mass • Stimulate endometrial growth • Increase uterine growth • Maintain blood vessels and skin

- Reduce bone resorption, increase bone formation

Protein synthesis:

- Increase hepatic production of binding proteins

Coagulation:

- Increase circulating level of factors 2, 7, 9, 10, antithrombin III, plasminogen
- Increase platelet adhesiveness
- Increase HDL, triglyceride, height growth
- Decrease LDL, fat deposition

Fluid balance:

- Regulate salt (sodium) and water retention
- Increase growth hormone
- Increase cortisol, SHBG

Gastrointestinal tract:

- Reduce bowel motility
- Increase cholesterol in bile

Melanin:

- Increase pheomelanin, reduce eumelanin

Cancer:

- Support hormone-sensitive breast cancers (Suppression of production in the body of estrogen is a treatment for these cancers.)

Lung function:

- Promote lung function by supporting

			alveoli.
Inhibin	Granulosa cells		Inhibit production of FSH from anterior pituitary

Placenta (when pregnant)

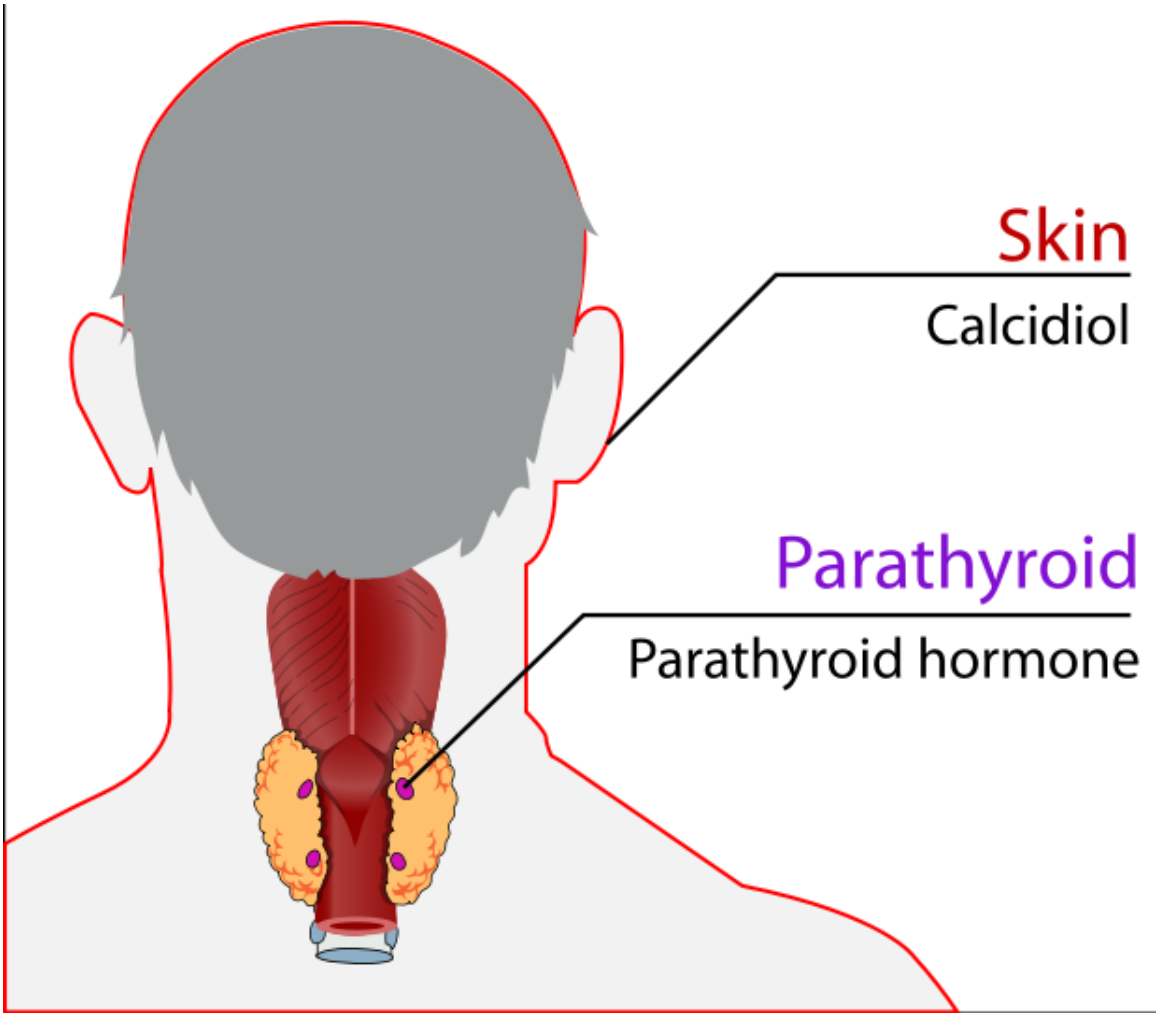
Secreted hormone	Abbreviation	From cells	Effect
Progesterone (Primarily)			Support pregnancy: • Inhibit immune response, towards the fetus. • Decrease uterine smooth muscle contractility • Inhibit lactation • Inhibit onset of labor. • Support fetal production of adrenal mineralo- and glucosteroids.
Estrogens (mainly Estriol) (Also Primarily)			Other effects on mother similar to ovarian follicle-progesterone Effects on mother similar to ovarian follicle estrogen
Human chorionic gonadotropin	HCG	Syncytiotrophoblast	promote maintenance of corpus luteum during beginning of pregnancy Inhibit immune response, towards the human embryo.
Human placental lactogen	HPL	Syncytiotrophoblast	increase production of insulin and IGF-1 increase insulin resistance and carbohydrate intolerance
Inhibin		Fetal Trophoblasts	suppress FSH

Uterus (when pregnant)

Secreted hormone	Abbreviation	From cells	Effect
Prolactin	PRL	Decidual cells	milk production in mammary glands
Relaxin		Decidual	Unclear in humans and animals

cells

Calcium regulation



Parathyroid

Secreted hormone	Abbreviation	From cells	Effect
Parathyroid hormone	PTH	Parathyroid chief cell	Calcium: • Stimulates Ca^{2+} release from bone, thereby increasing blood Ca^{2+} • Stimulates osteoclasts, thus breaking down bone • Stimulates Ca^{2+} reabsorption in kidney • Stimulates activated vitamin D

production in kidney

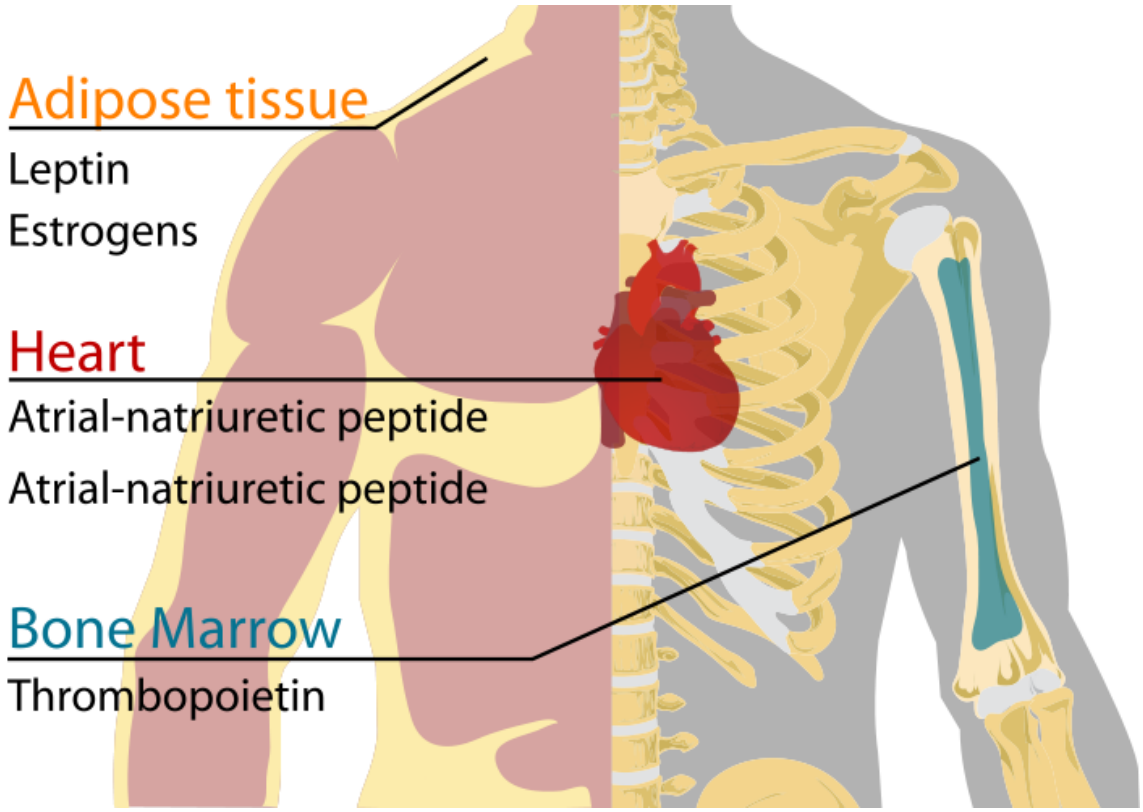
Phosphate:

- Stimulates PO^4 release from bones, thereby increasing blood PO^4.
- Inhibits PO^4 reabsorption in kidney, so more PO^4 = is excreted
- Overall, small net drop in serum PO^4.

Skin

Secreted hormone	From cells	Effect
Calcidiol (25-hydroxyvitamin D_3)		Inactive form of vitamin D_3

Miscellaneous



Heart

Secreted hormone	Abbreviation	From cells	Effect
Atrial-natriuretic peptide	ANP	Cardiac myocytes	Reduce blood pressure by: reducing systemic vascular resistance, reducing blood water, sodium and fats
Brain natriuretic peptide	BNP	Cardiac myocytes	(To a lesser degree than ANP) reduce blood pressure by: reducing systemic vascular resistance, reducing blood water, sodium and fats

Bone Marrow

Secreted hormone	From cells	Effect
Thrombopoietin	liver and kidney cells	stimulates megakaryocytes to produce platelets

Adipose tissue

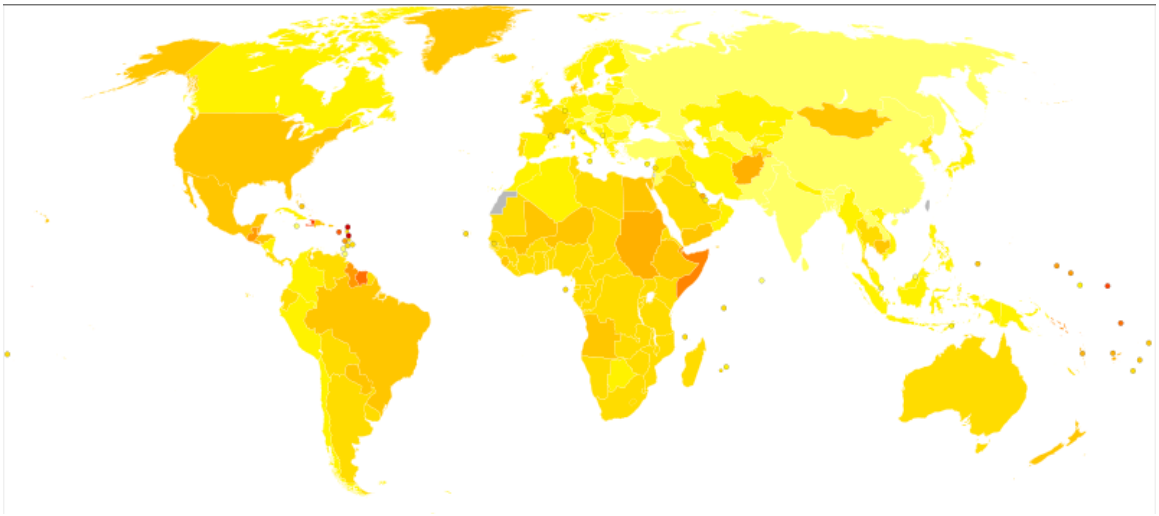
Secreted hormone	From cells	Effect
Leptin (Primarily)	Adipocytes	decrease of appetite and increase of metabolism.
Estrogens (mainly Estrone)	Adipocytes	

Major endocrine systems

The human endocrine system consists of several integrated systems that operate via feedback loops. Several important feedback systems are mediated via the hypothalamus and pituitary.

- TRH - TSH - T3/T4
- GnRH - LH/FSH - sex hormones
- CRH - ACTH - cortisol
- Renin - angiotensin - aldosterone

Diseases



Disability-adjusted life year for endocrine disorders per 100,000 inhabitants in 2002.
- no data
- less than 80
- 80-160
- 160-240
- 240-320
- 320-400
- 400-480
- 480-560
- 560-640
- 640-720
- 720-800
- 800-1000
- more than 1000

Diseases of the endocrine system are common, including conditions such as diabetes mellitus, thyroid disease, and obesity. Endocrine disease is characterized by disregulated hormone release (a productive pituitary adenoma), inappropriate response to signaling (hypothyroidism), lack of a gland (diabetes mellitus type 1, diminished erythropoiesis in chronic renal failure), or structural enlargement in a critical site such as the testis (toxic multinodular goitre). Hypofunction of endocrine glands can occur as a result of loss of reserve, hyposecretion, agenesis, atrophy, or active destruction. Hyperfunction can occur as a result of hypersecretion, loss of suppression, hyperplastic or neoplastic change, or hyperstimulation.

Endocrinopathies are classified as primary, secondary, or tertiary. Primary endocrine disease inhibits the action of downstream glands. Secondary endocrine disease is indicative of a problem with the pituitary gland. Tertiary endocrine disease is associated with dysfunction of the hypothalamus and its releasing hormones.

As the thyroid, and hormones have been implicated in signaling distant tissues to proliferate, for example, the estrogen receptor has been shown to be involved in certain breast cancers. Endocrine, paracrine, and autocrine signaling have all been implicated in proliferation, one of the required steps of oncogenesis.

Other types of signaling

The typical mode of cell signaling in the endocrine system is endocrine signaling. However, there are also other modes, i.e., paracrine, autocrine, and neuroendocrine signaling. Purely neurocrine signaling between neurons, on the other hand, belongs completely to the nervous system.

Autocrine

Autocrine signaling is a form of signaling in which a cell secretes a hormone or chemical messenger (called the autocrine agent) that binds to autocrine receptors on the same cell, leading to changes in the cells.

Paracrine

Paracrine signaling is a form of cell signaling in which the target cell is near the signal-releasing cell.

Juxtacrine

juxtacrine signaling is a type of intercellular communication that is transmitted via oligosaccharide, lipid, or protein components of a cell membrane, and may affect either the emitting cell or the immediately adjacent cells.

It occurs between adjacent cells that possess broad patches of closely opposed plasma membrane linked by transmembrane channels known as connexons. The gap between the cells can usually be between only 2 and 4 nm.

Unlike other types of cell signaling (such as paracrine and endocrine), juxtacrine signaling requires physical contact between the two cells involved.

Chapter- 9

Cell Surface Receptor

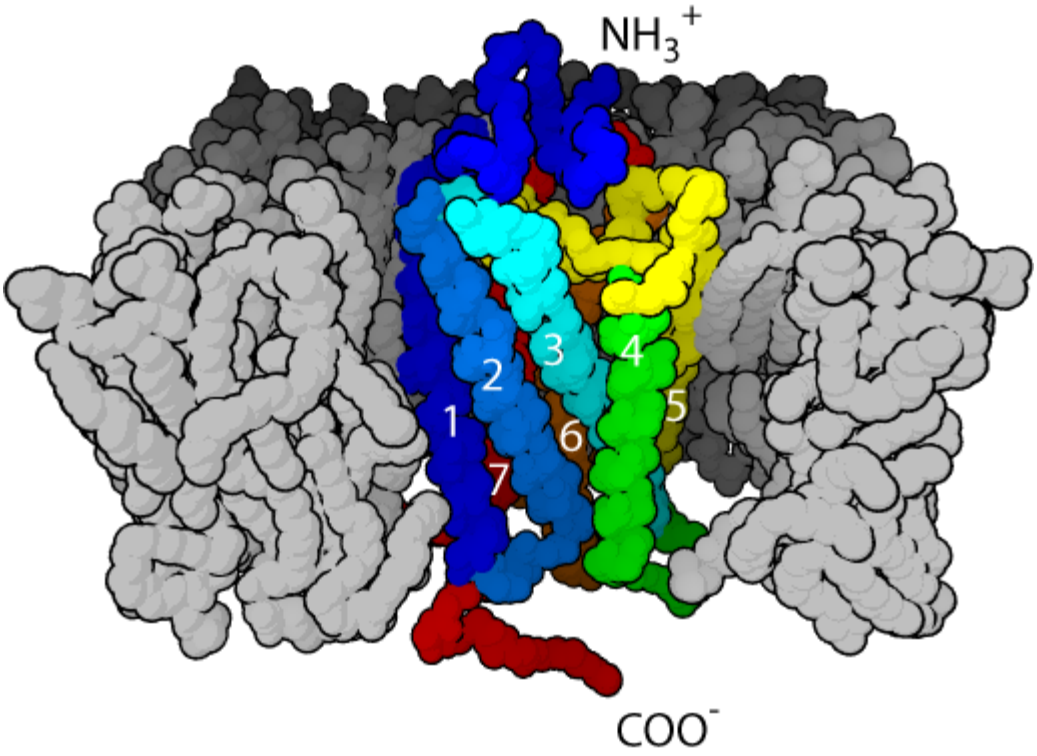


Figure 1. The seven-transmembrane α-helix structure of a G-protein-coupled receptor.

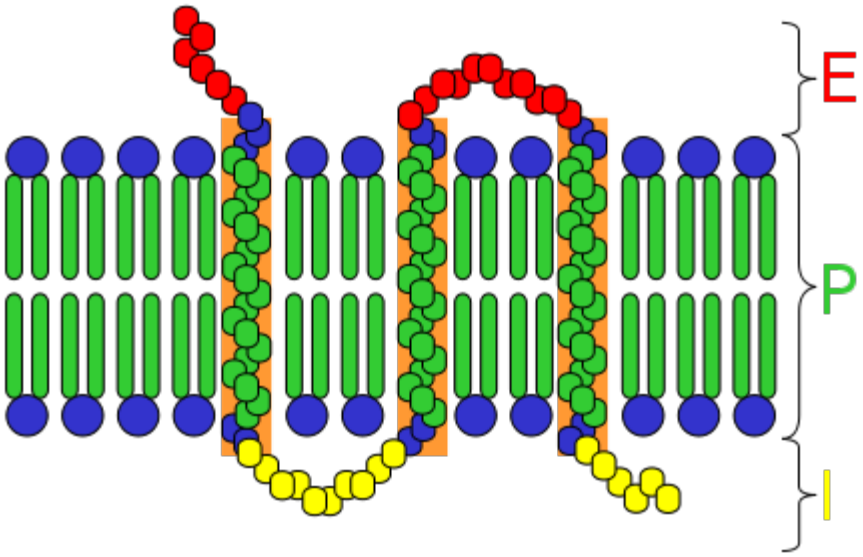
Cell surface receptors (**membrane receptors**, **transmembrane receptors**) are specialized integral membrane proteins that take part in communication between the cell and the outside world. Extracellular signaling molecules (usually hormones, neurotransmitters, cytokines, growth factors or cell recognition molecules) attach to the receptor, triggering changes in the function of the cell. This process is called signal transduction: The binding initiates a chemical change on the intracellular side of the membrane. In this way the receptors play a unique and important role in cellular communications and signal transduction.

Structure and mechanism

Many transmembrane receptors are composed of two or more protein subunits which operate collectively and may dissociate when ligands bind, fall off, or at another stage of their "activation" cycles. They are often classified based on their molecular structure, or because the structure is unknown in any detail for all but a few receptors, based on their hypothesized (and sometimes experimentally verified) membrane topology. The polypeptide chains of the simplest are predicted to cross the lipid bilayer only once, while others cross as many as seven times (for example, the so-called G-protein coupled receptors).

There are various kinds, such as glycoprotein and lipoprotein. Hundreds of different receptors are known and many more are yet to be discovered. Almost all known membrane receptors are transmembrane proteins. A certain cell membrane can have several membrane receptors with various amounts on its surface. A certain receptor may also exist at varying concentrations on different membrane surfaces, depending on the membrane and cell function. Since receptors usually form “clusters” on the membrane surface, the distribution of receptors on membrane surface is mostly heterogeneous.

Domains



E = extracellular space
P = plasma membrane
I = intracellular space

Like any integral membrane protein, a transmembrane receptor may be subdivided into three parts or *domains*.

Extracellular domain

The extracellular domain is the part of the receptor that sticks out of the membrane on the outside of the cell or organelle. If the polypeptide chain of the receptor crosses the bilayer several times, the external domain can comprise several "loops" sticking out of the membrane. By definition, a receptor's main function is to recognize and respond to a specific ligand, for example, a neurotransmitter or hormone (although certain receptors respond also to changes in transmembrane potential), and in many receptors these ligands bind to the extracellular domain.

Transmembrane domain

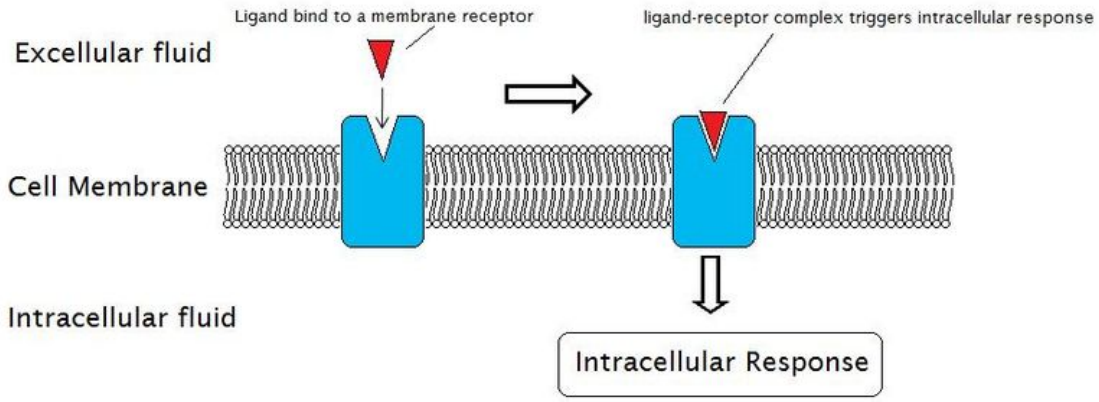
In the majority of receptors for which structural evidence exists, transmembrane alpha helices make up most of the transmembrane domain. In certain receptors, such as the nicotinic acetylcholine receptor, the transmembrane domain forms a protein-lined pore through the membrane, or ion channel. Upon activation of an extracellular domain by binding of the appropriate ligand, the pore becomes accessible to ions, which then pass through. In other receptors, the transmembrane domains are presumed to undergo a conformational change upon binding, which exerts an effect intracellularly. In some receptors, such as members of the 7TM superfamily, the transmembrane domain may contain the ligand binding pocket (evidence for this and for much of what else is known about this class of receptors is based in part on studies of bacteriorhodopsin, the detailed structure of which has been determined by crystallography).

Intracellular domain

The intracellular (or cytoplasmic) domain of the receptor interacts with the interior of the cell or organelle, relaying the signal. There are two fundamentally different ways for this interaction:

- The intracellular domain communicates via specific protein-protein-interactions with *effector proteins*, which in turn send the signal along a signal chain to its destination.
- With enzyme-linked receptors, the intracellular domain has *enzymatic activity*. Often, this is a tyrosine kinase activity. The enzymatic activity can also be located on an enzyme associated with the intracellular domain.

Signal transduction

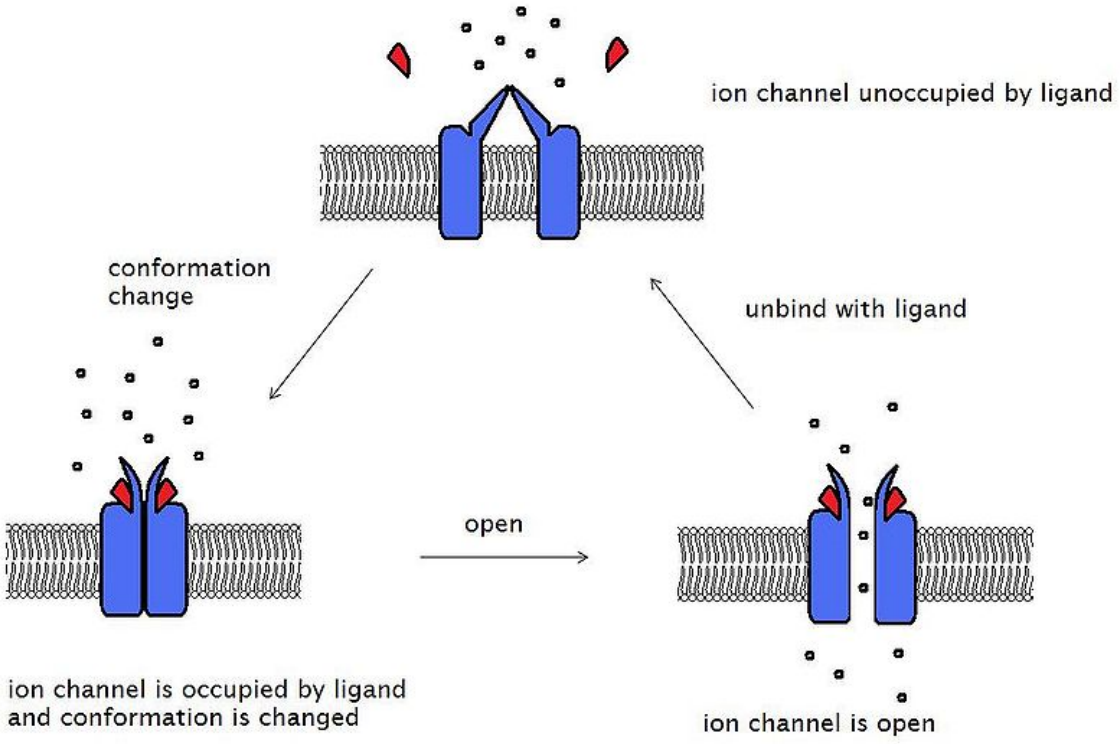


External reactions and internal reactions for signal transduction

Signal transduction processes through membrane receptors involve the External Reactions, in which the ligand binds to a membrane receptor, and the Internal Reactions, in which intracellular response is triggered.

Signal transduction through membrane receptors usually requires four characters:

- Extracellular signal molecule: an extracellular signal molecule is produced by one cell and is capable of traveling to neighboring cells, or to cells that may be far away.
- Receptor protein: the cells in an organism must have cell surface receptor proteins that bind to the signal molecule and communicate its presence inward into the cell.
- Intracellular signaling proteins: these distribute the signal to the appropriate parts of the cell. The binding of the signal molecule to the receptor protein will activate intracellular signaling proteins that initiate a signaling cascade (a series of intracellular signaling molecules that act sequentially).
- Target proteins: the conformations or other properties of the target proteins are altered when a signaling pathway is active and changes the behavior of the cell.



Three conformation states of acetylcholine receptor

Based on structural and functional similarities, membrane receptors are mainly divided into 3 classes: The ion channel-linked receptor; The enzyme-linked receptor and G protein-coupled receptor.

- **Ion channel-linked receptors** are ion-channels (including cation-channels and anion-channels) themselves and constitute a large family of multipass transmembrane proteins. They are involved in rapid signaling events most generally found in electrically excitable cells such as neurons and are also called ligand-gated ion channels. Opening and closing of Ion channels are controlled by neurotransmitters.

- **Enzyme-linked receptors** are either enzymes themselves, or are directly associated with the enzymes that they activate. These are usually single-pass transmembrane receptors, with the enzymatic portion of the receptor being intracellular. The majority of enzyme-lined receptors are protein kinases, or associate with protein kinases.

- **G protein-coupled receptors** are integral membrane proteins that possess seven membrane-spanning domains or transmembrane helices. These receptors activate a G protein ligand binding. G-protein is a trimeric protein. The 3 subunits are called α、β and γ. The α subunit can bind with guanosine diphosphate, GDP. This causes phosphorylation of the GDP to guanosine triphosphate, GTP, and activates the α subunit, which then dissociates from the β and γ subunits. The activated α

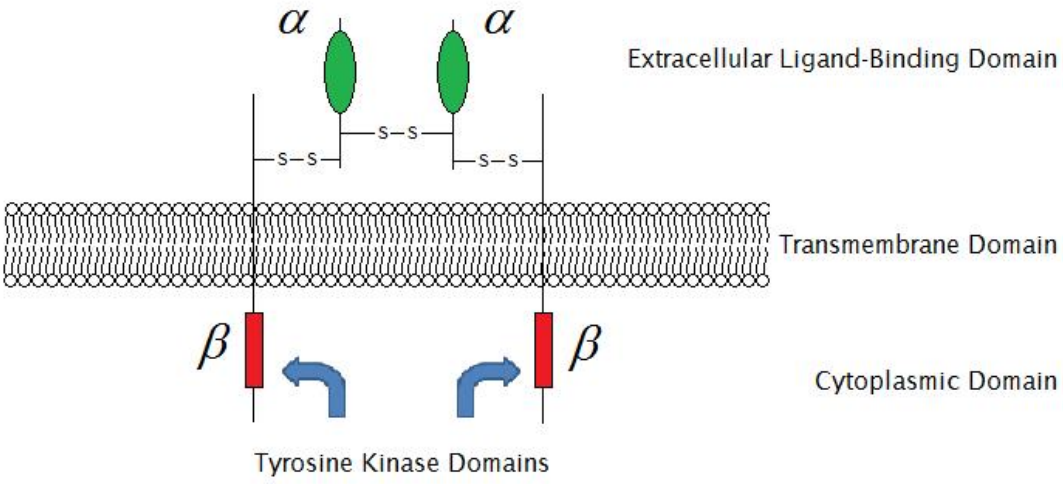
subunit can further affect intracellular signaling proteins or target functional proteins directly.

Ion channel-linked receptor

In the signal transduction event in a neuron, the neurotransmitter binds with the receptor and alters the conformation of the protein, which opens the ion-channel, allowing extracellular ions go into the cell. The ion permeability of the plasma membrane is altered, and this will instantaneously convert the extracellular chemical signal into intracellular electric signal, which will alter the excitability of the cell.

Acetylcholine receptor is a kind of cation-channel linked receptor. The protein consists of 4 subunits: α, β, γ, and δ subunits. There are two α subunits, containing one acetylcholine binding site each. This receptor can exist in three different conformations. The unoccupied-closed state is the protein at its original conformation. After two molecules of acetylcholine bind simultaneously to the binding sites on α subunits, the conformation of the receptor is altered and the gate is opened, allowing for the penetration of many ions and small molecules. However, this occupied-open state can only last for a very short period of time and then the gate is closed again, forming the occupied-closed state. The two molecules of acetylcholine will quickly dissociate from the receptor and the receptor will returns to its unoccupied-closed state and is ready for next transduction cycle again.

Enzyme-linked receptors



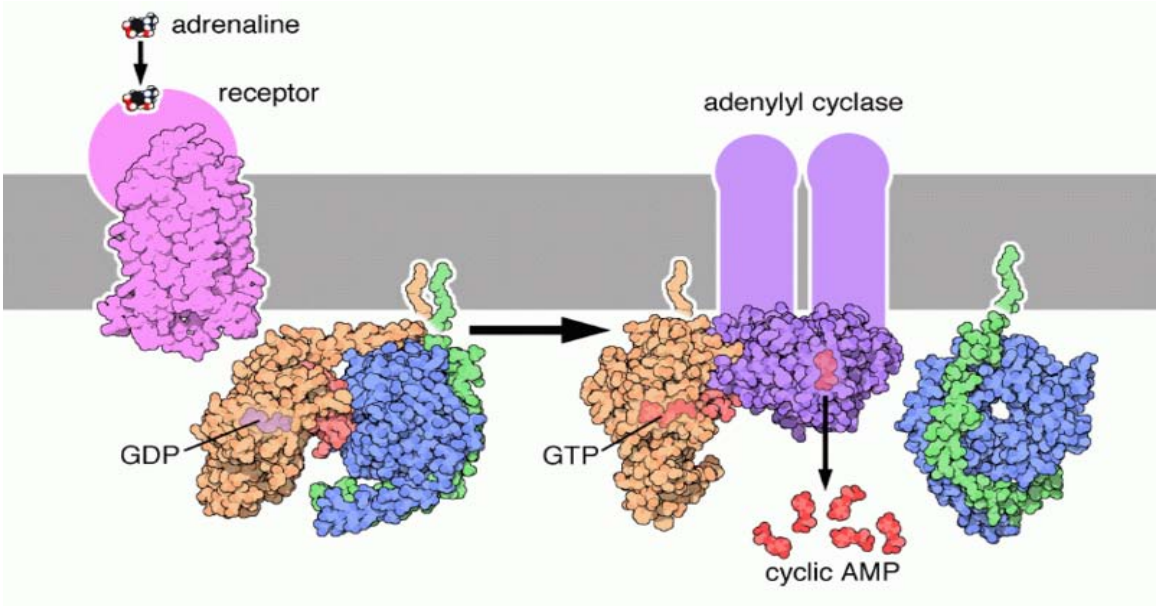
Sketch of an enzyme-linked receptor structure (structure of IGF-1R)

As of 2009, there are 6 known types of enzyme-linked receptors: Receptor tyrosine kinases; Tyrosine kinase associated receptors; Receptor-like tyrosine phosphatases; Receptor serine/threonine kinases; Receptor Guanylyl cyclases and Histidine kinase associated receptors. Receptor tyrosine kinases is the one kind with the largest population

and most widely application. The majority of these molecules are receptors for growth factors and hormones like epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin, nerve growth factor (NGF) etc.

Most of these receptors will dimerize after binding with their ligands in order to activate further signal transductions. For example, after the epidermal growth factor (EGF) receptor binds with its ligand EGF, two receptors dimerize and then undergo phosphorylation of the tyrosine residues in the enzyme portion of each receptor molecule, which will activate the tyrosine protein kinase and analyze further intracellular reactions.

G protein-linked receptors



Epinephrine binds its receptor, that associates with an heterotrimeric G protein. The G protein associates with adenylate cyclase that converts ATP to cAMP, spreading the signal

G protein-coupled receptors comprise a large protein family transmembrane receptors. They are found only in eukaryotes. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases, and are also the target of around half of all modern medicinal drugs.

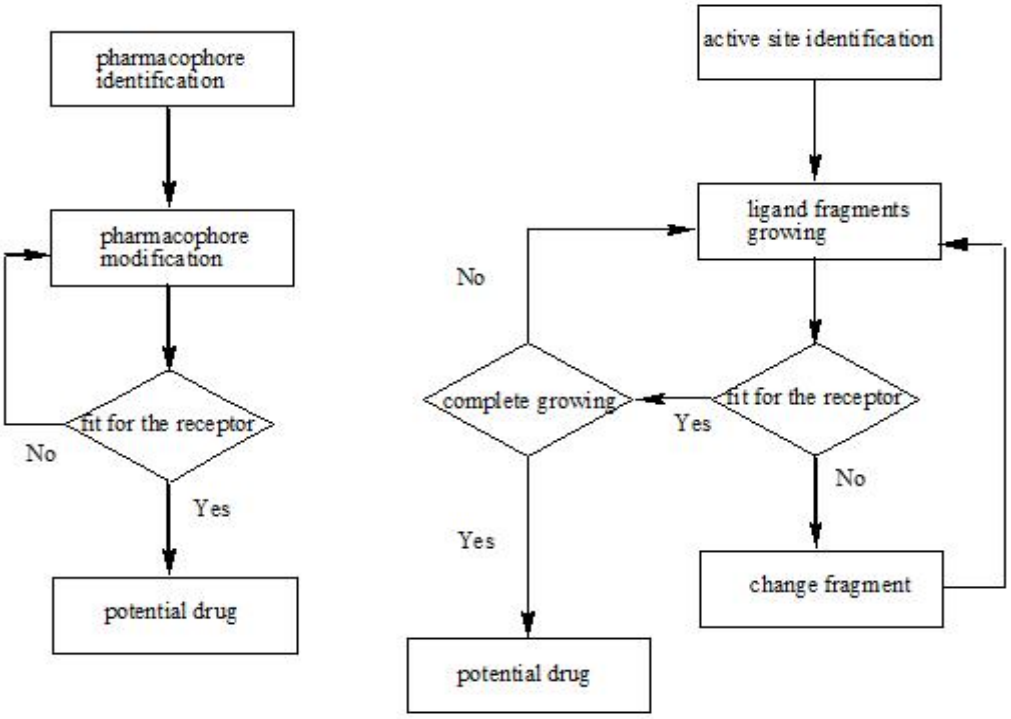
There are two principal signal transduction pathways involving the G-protein coupled receptors: cAMP signal pathway and Phosphatidylinositol signal pathway. Both activate a G protein ligand binding. G-protein is a trimeric protein. The 3 subunits are called α、β and γ. The α subunit can bind with guanosine diphosphate, GDP. This causes phosphorylation of the GDP to guanosine triphosphate, GTP, and activates the α subunit,

which then dissociates from the β and γ subunits. The activated α subunit can further affect intracellular signaling proteins or target functional proteins directly.

Membrane receptor-related disease

If the membrane receptors are altered directly or deficient for some reason, the signal transduction can be hindered and cause diseases. Some diseases are caused by membrane receptor function disorder due to deficiency or disorder of the receptor induced by the change in the genes that encode the receptor protein. Scientists recently have found that the membrane receptor TM4SF5 has something to do with the migration ability of hepatic cells and hepatoma. and that the cortical NMDA receptor properties and membrane fluidity are altered in Alzheimer's disease. Also, when the cell is infected by nonenveloped virus, the virus first binds with certain membrane receptors and then somehow the virus or some subviral component ends up on the cytoplasmic side of a cellular membrane, the plasma membrane for some viruses or the membrane of an endosomal vesicle for others. In the case of poliovirus, it is known that interactions with receptors in vitro will lead to conformational rearrangements of the virion that result in the release of one of the virion proteins, called VP4.The N terminal of VP4 is myristylated and thus hydrophobic【myristic acid=$CH_3(CH_2)_{12}COOH$】. It is proposed that the conformational changes induced by receptor binding result in the insertion of the myristic acid on VP4 into the cell membrane and the formation of a channel through which the RNA can enter the cell.

Structure-based drug design



Flow charts of two strategies of structure-based drug design

As methods such as X-ray crystallography and NMR spectroscopy develop, the amount of information about 3D structures of biomolecular targets has increased dramatically, as well as the structural dynamic and electronic information about the ligands. This stimulates rapid development of structure-based drug design. Some of these new drugs target membrane receptors. Current methods for structure-based drug design can be divided roughly into two categories. The first category is about “finding” ligands for a given receptor. This is usually referred to as database searching. In this case, a large number of potential ligand molecules are screened to find those fitting the binding pocket of the receptor. This method is usually referred to as ligand-based drug design. The key advantage of database searching is that it saves synthetic effort to obtain new lead compounds. Another category of structure-based drug design methods is about “building” ligands, which is usually referred to as receptor-based drug design. In this case, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either atoms or fragments. The key advantage of such a method is that novel structures, not contained in any database, can be suggested.