Cell signalling

Cell signaling refers to **communication between cells**.

Regulates:

- the development of cells and their organization into tissues
- cell growth and cell division
- coordination of cellular functions

Signalling types

Endocrine (hormonal)

Cells secrete signalling molecules (primarily hormones), that enter the blood and the circulatory system to the target cell, to which the receptor binds.

- Action – remote
- Speed – in minutes

Paracrine

The cells release chemical substances (e.g. growth factors) into the ECF, which act as local mediators and affect the cells in the immediate vicinity. For example, signalling molecules that regulate inflammation at the site of infection or cell proliferation during wound healing work in this way.

Autocrine

The secreted signalling molecule binds to the receptor of the cell that produced it.

Synaptic

This signalling is specific to the nervous system of animals. A nerve cell produces a chemical signal (neurotransmitter), that is transmitted to another nerve cell through synaptic cleft.

- Speed − up to 100 m/s[1].

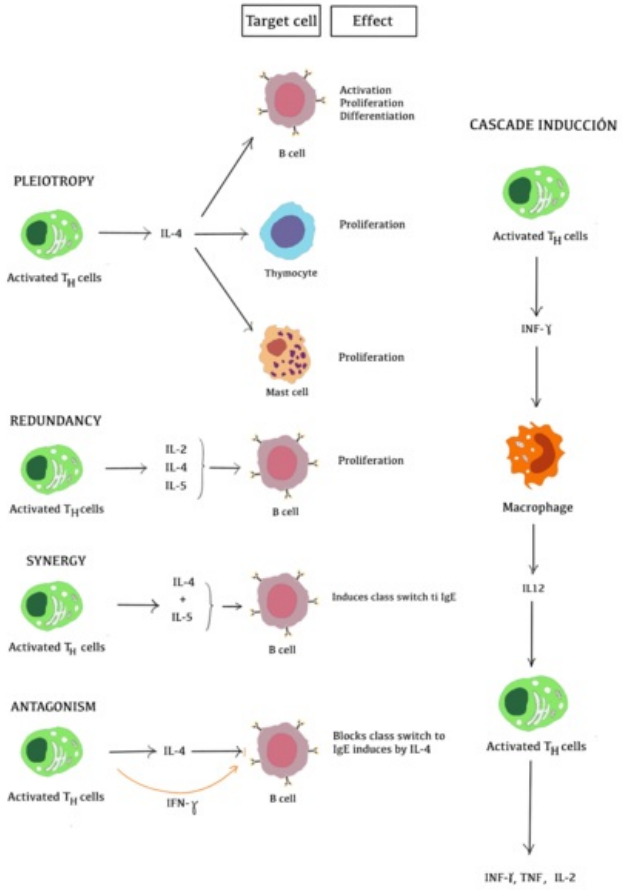
Ways of cellular communication

By direct contact

1. Junctional complexes ensure the continuity of adjacent cells. In animal cells using nexus (gap junctions), in plant cells using plasmodesmata.
2. Interaction of cell surface molecules.

Through signalling molecules

Signal molecules are substances that are capable of transmitting a signal. They can be divided according to their chemical nature into several groups: e.g. cytokine interactions between cells of the immune system



1. **Lipophilic signalling molecules**:
   - steroid hormones,
   - thyroid hormones,
   - fatty acid derivatives (eicosanoids),
   - retinoids (retinal).
2. **Peptide and protein signalling molecules**:
   - peptide hormones (e.g., liberins, statins, insulin, glucagon, vasopressin),
   - growth factors and cytokines.
3. **Amino acid derivatives**:
   - hormones (e.g., adrenaline, noradrenaline),
   - neurotransmitters (e.g., GABA, glutamate, glycine),
   - mediators (e.g., histamine).
4. **Malé anorganické molekuly a ionty**:
   - NO,
   - CO,
   - Ca^{2+}.
5. **Nukleotides**:
   - cAMP,
   - cGMP.

Mechanism of action of signalling molecules

- It depends on whether the signaling molecule is water soluble (hydrophilic) or fat soluble (hydrophobic).
- Cytoplasmic membrane of cells is permeable to hydrophobic (= lipophilic) signal molecules and to small inorganic molecules such as NO. These molecules bind to **cytoplasmic or nuclear receptors**, which mostly act as ligand-directed transcription factors and, after binding the signalling molecule, affect gene transcription.
- For hydrophilic signalling molecules (peptides, proteins) and amino acid derivatives, the cytoplasmic membrane is impermeable and therefore their signalling must take place via receptors located in the cytoplasmic membrane of the target cells (so-called **membrane receptors**). After binding a signal molecule (ligand) to a membrane receptor, signal transduction occurs; signal transmission from the receptor to the interior of the cell. Intracellular signalling follows, often involving second messengers or specific protein kinases. These subsequently regulate the activity of effector proteins and the behaviour of the cell will change. Effector proteins can be enzymes affecting metabolism, transcription factors, components of the cytoskeleton or ion channels.

Signalling stages

1. Production of a signalling molecule by the cell (based on superior stimulation – e.g. hormones controlled by the Hypothalamic-pituitary-gonadal axis, or when the concentration of certain molecules changes – e.g., glucose or ions).
2. Reception of the signal by the target cell → the signalling molecule binds to the receptor.
3. Signal transmission (= signal transduction) – can be one-step or involve a cascade of changes in molecules (the so-called signalling pathway).
4. A signal triggers a specific response.
5. Degradation of the signalling molecule.

Types of membrane receptors

They differ in the signal that is created inside the cell after the binding of the extracellular signal molecule to the receptor.
⚠ **Only hydrophilic substances bind to membrane receptors. Hydrophobic substances pass through the membrane without specific carriers and bind to their receptors only in the cytoplasm or in the nucleus (the best-known hydrophobic substances are steroids and thyroid hormones).**

Enzymotropic receptors (catalytic receptors, enzyme-linked receptors or receptors with intrinsic enzymatic activity)

- Some proteins pass through the phospholipid bilayer of the membrane only once. They consist of an extracellular part of a protein with a binding site for a signalling molecule, a transmembrane α-helix, and a cytoplasmic part that either contains its enzymatic activity or is associated with an enzyme.
- A receptor is either a ligand-directed enzyme or a protein that binds to the enzyme. Many receptors contain a cytoplasmic portion that functions as a tyrosine-protein kinase.
- After binding the signal molecule, 2 receptor proteins join and a dimer is formed. This activates the tyrosine kinase parts of the receptor, which phosphorylate tyrosine (using phosphate groups from ATP) of the receptor itself.
- Phosphorylated tyrosines serve as binding sites for various proteins, which themselves become active after binding.
- For example, the signalling molecule Ras (GTP-binding protein) is activated, which subsequently activates other protein kinases. As a consequence, a change in gene expression occurs. Signal termination is catalyzed by protein-tyrosine-phosphatase, or activated receptors can undergo Endocytosis and be degraded by Lysosomes.
    - E.g., growth factor or insulin, bind to receptors with tyrosine kinase activity (more detail below).
- The group of enzymotropic receptors also includes receptors with serine/threonine kinase, guanylate cyclase or tyrosine phosphatase activity.

Receptors with tyrosine kinase activity

They are predominantly receptors for most growth and differentiation factors such as **EGF** (epidermal growth factor), **PDGF** (platelet-derived growth factor), **IGF-1** (insulin-like growth factor) and the insulin receptor. After the binding of the ligand to the receptor, it is activated and the phosphate group is transferred from **ATP** to specific **tyrosines**. Either the tyrosines of the receptor proteins themselves (autophosphorylation) or the tyrosines of specific cellular proteins (intracellular protein kinases) are phosphorylated. This initiates a cascade of intracellular signal transmission.

Ras proteins are among the important intracellular signal proteins that are primarily involved in **signal transmission from a receptor with tyrosine kinase activity to the interior of the cell**, where they activate the serine/threonine phosphorylation cascade. Ras proteins are anchored in the cytoplasmic part of the plasma membrane. It belongs to the family of **monomeric GTPases** (**as opposed to G proteins – trimeric GTPases**). However, the activation and function of monomeric and trimeric GTPases are similar. They are in a constant transition between an **active state** when **GTP** is bound to them, and an **inactive state** when **GDP** is bound. Ras proteins are phosphorylated (activated) by receptor tyrosine kinases and inactivated by phosphatases and GTP hydrolysis, which they carry out.

The **tyrosine phosphorylation of Ras proteins**, which is carried out by receptor tyrosine kinases on the cytoplasmic side of the plasma membrane, is soon terminated by dephosphorylation by specific **tyrosine phosphatases**. Activated Ras proteins also inactivate themselves by hydrolysis of bound **GTP** to **GDP**. Stimulating cells to proliferate and differentiate, however, requires long-term signalling. Further signal transmission is ensured by **phosphorylation of serines and threonines by MAP-protein kinases (mitogen-activated protein kinases)** . Phosphorylation of serines and threonines has a longer duration than **tyrosine phosphorylation of Ras proteins.**

The active Ras/GTP complex binds to Raf-kinase (MAP-kinase 1) and activates it by phosphorylating serines and threonines.

Other protein kinases are also involved in the regulation of Raf-kinase activity:

1. activation of Raf-protein kinase increases Src-protein kinase by tyrosine phosphorylation
2. protein kinase C by serine phosphorylation

3. serine phosphorylation by protein kinase A has an inhibitory effect

Active **Raf-kinase** activates **MAP-kinase 2** by phosphorylation, which activates **MAP-kinase 3**, which enters the nucleus. Here, a regulatory protein is activated, which stimulates the activity of genes involved mainly in the regulation of cell proliferation.

Activated **Ras protein** phosphorylate and thereby **activate a cascade of three types of MAP-kinases**. Binding of the first MAP-kinase (referred to as Raf) to the activated Ras protein results in its phosphorylation and thus activation. This then catalyzes serine/threonine phosphorylation of another MAP-kinase, and this enzyme activates another (third) MAP-kinase. Activation of the last MAP-kinase in the cascade by MAP-kinase phosphorylation requires phosphorylation of both **threonine** and **tyrosine**. After entering the nucleus, the third MAP-kinase activated in this way **first phosphorylates the regulatory protein**, which is bound to a short DNA sequence in the regulatory region of the early response genes - the **myc, jun a fos** genes. This results in their transcription.

Late-response gene products are involved in the regulation of cell proliferation. These include, for example, the main components of the cell cycle control system – **cyclins and cyclin-dependent protein kinases**.

Receptors with tyrosine phosphatase activity

The specific activity of these enzymes ensures that the phosphorylation of tyrosines takes a **very short time**and that only a **small amount**of tyrosines are phosphorylated in resting cells. An example of a receptor with tyrosine phosphatase activity is the membrane **glycoprotein CD45**, which is found on the surface of white blood cells. It participates in the activation of T and B lymphocytes after encountering foreign antigens.

Receptors with guanylate cyclase activity

For example, a receptor binding **atrial natriuretic peptides** (ANPs) is a group of peptide hormones. They are found in kidney cells and in **smooth muscle cells of blood vessels**. Atrial natriuretic peptides are secreted by **atrial muscle cells** when blood pressure rises. They stimulate the kidneys to excrete Na+ and water and induce the relaxation of muscle cells in the walls of blood vessels. Both of these effects lead to a reduction in blood pressure.

The receptors have an extracellular region for binding ANPs and an intracellular guanylate cyclase catalytic domain. The binding of the ligand to the receptor activates the cyclase to produce cyclic 3',5'-GMP (cGMP). cGMP binds to cGMP-dependent protein kinase and thus activates it to phosphorylate the serines and threonines of specific proteins, which are involved in further signal transmission and the realisation of the final expression.

Receptors with attached tyrosine kinase activity

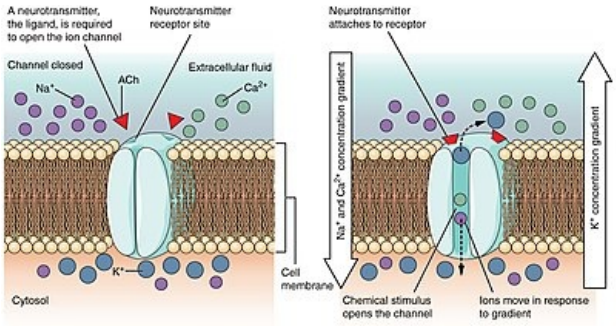
It differs from receptors with tyrosine kinase activity in that the tyrosine kinase in this case is encoded by another separate gene (e.g. proto-oncogene Src) and is non-covalently attached to the cytoplasmic part of the receptor polypeptide chain. These receptors form a large heterogeneous group. They are, for example, receptors for most **cytokines**that regulate the proliferation and differentiation of cells of the hemopoietic system; antigen-specific receptors on T and B lymphocytes; hormone receptors (eg growth hormone, prolactin) and others.

The antigen is presented by **MHC** molecules and recognized by T lymphocyte receptors (TCR); The TCR is activated and transmits a signal via signalling molecules to the nucleus. **Cytokine** are subsequently expressed.

The secreted cytokine binds to and activates a B lymphocyte membrane receptor with associated **tyrosine kinase activity**. The tyrosine kinase is encoded by the **proto-oncogene Src**.

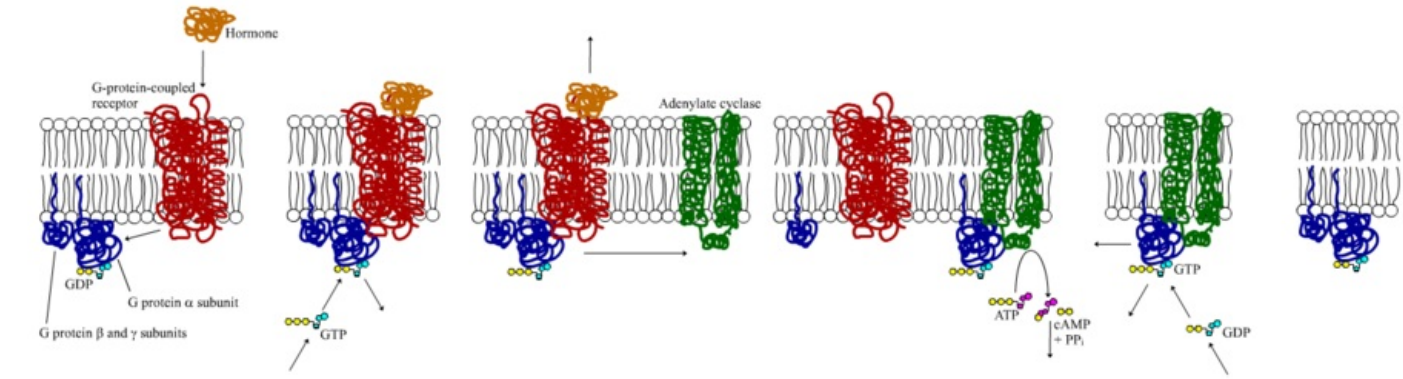
Receptors associated with ion channels (ionotropic receptors)

Some receptor proteins regulate the activity of ion channels by binding to a signalling molecule. Their opening and closing is their signalling response. After the binding of the nerve mediator, the conformation of the receptor changes and the ion channel closes or, conversely, opens for specific ions that move along their electrochemical gradient and the membrane potential changes. This type of cellular signalling occurs in so-called excitable tissues - the nervous system and muscles.



G-protein-coupled receptors (GPCR = G-protein–coupled receptor)

A receptor is a polypeptide chain that crosses the membrane seven times. In the resting state, the G-protein probably does not even touch the receptor. It consists of three subunits α, β, γ. GDP is bound to the α subunit at rest. After ligand binding, the receptor couples to a G-protein and GDP is replaced by GTP. Termination of the signal is accompanied by the hydrolysis of GTP back to GDP (the α subunit has *GTPase activity*). The target of action of the activated G-protein (its dissociated α subunit or βγ complex) can be ion channels or enzymes in the membrane. ***Adenylate cyclase*** (production of cAMP) and ***Phospholipase C*** (production of IP_3 and DAG) are most often activated.



General diagram of the signalling pathway

Hormone → membrane receptor → G-protein → adenylate cyclase → cAMP → protein kinase A →

1. phosphorylation of enzymes affecting metabolism (rapid effects)
2. phosphorylation of gene regulatory proteins → influencing gene transcription (slow effects)

Second messengers and their functions

Second messengers are small, non-protein, water-soluble molecules. They are called second because the first messenger is a signalling molecule that binds to a receptor protein. They are part of signal transduction in pathways initiated by both G-protein coupled receptors and enzymotropic receptors. They arise from easily accessible substrates and have a short biological half-life. Second messengers include cAMP, **calcium cations, cGMP, inositol-1,4,5-trisphosphate, diacylglycerol, phosphatidylinositol-3,4,5-trisphosphate** and others. Except for calcium cations, second messengers are synthesized by specific enzymes after stimulation of membrane receptors. They subsequently activate protein kinases that phosphorylate serine amino acids or threonine in various intracellular proteins. Phosphorylation changes the activity of these proteins in terms of activation or inhibition. The activity of second messengers is limited and they are degraded by various enzymes.

The second messenger	Substrate	Enzyme	Effector	Degradation
cAMP	ATP	adenylate cyclase (AC)	protein kinase A (PKA)	phosphodiesterases (PDE)
cGMP	GTP	guanylate cyclase (GC)	protein kinase G (PKG)	phosphodiesterases (PDE)
calcium cations (Ca^{2+})	–	release from ER after IP_3 stimulation	calmodulin	reabsorption into the ER by Ca^{2+} ATPase
inositol-1,4,5-trisphosphate (IP_3)	phosphatidylinositol-4,5-bisphosphate (PIP_2)	phospholipase C (PLC)	protein kinase C (PKC)	phosphatases – dephosphorylation to inositol
diacylglycerol (DAG)	phosphatidylinositol-4,5-bisphosphate (PIP_2)	phospholipase C (PLC)	protein kinase C (PKC)	lipase – formation of glycerol and free fatty acids
phosphatidylinositol-3,4,5-trisphosphate (PIP_3)	phosphatidylinositol-4,5-bisphosphate (PIP_2)	phosphatidylinositol-3-kinase (PI3K)	protein kinase B (PKB)	phosphatase PTEN – cleavage of phosphate in position 3

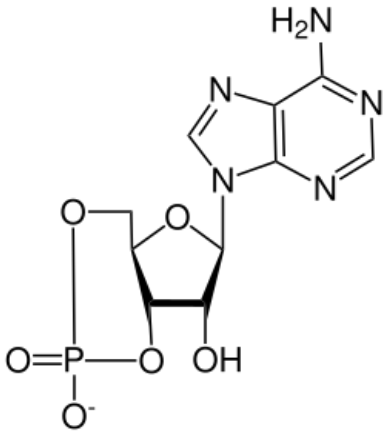
Cyclic adenosine monophosphate (cAMP)

Cyclic AMP is formed from ATP by the enzyme ***adenylate cyclase***, which is anchored in the cytoplasmic membrane and is activated via G-protein after binding of the signalling molecule to the membrane receptor. cAMP then transmits a signal from the cytoplasmic membrane to metabolic processes in the cytoplasm. The transfer molecule following cAMP is usually **protein kinase A (cAMP-dependent protein kinase)**, which phosphorylates other proteins. PKA-regulated proteins are involved, for example, in the control of carbohydrate and lipid metabolism, water and ion transport in the kidneys, etc. cAMP does not remain in the cell for long and is converted by phosphodiesterase into AMP (adenosine monophosphate).

Cyklický guanosinmonofosfát (cGMP)

It arises similarly to cAMP, i.e. it is formed by the enzyme ***guanylate cyclase*** from GTP. Guanylate cyclase activity is activated, for example, by atrial natriuretic peptide (ANP)[2]. cGMP activates **protein kinase G (cGMP-dependent protein kinase)**, which phosphorylates target proteins. Signal transduction involving cGMP is used,

for example, by rods in the retina of the eye or smooth muscle cells of the cavernous bodies of the penis.

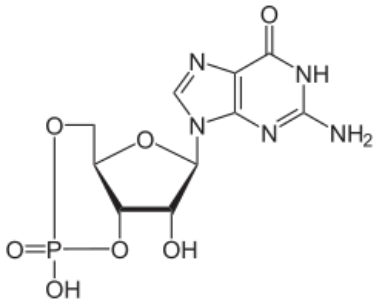


Second messenger derivation from phosphatidylinositol-4,5-bisphosphate

Phosphatidylinositol-4,5-bisphosphate (PIP_2, also $PtdIns(4,5)P_2$) is a phospholipid located in the cytoplasmic membrane. Second messengers can be synthesized from phosphatidylinositol-4,5-bisphosphate which affects 2 distinct protein kinases.

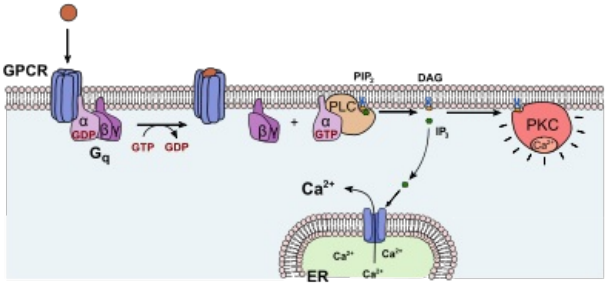
Phosphatidylinositol-3,4,5-trisphosphate

Phosphatidylinositol-3,4,5-trisphosphate (PIP_3, also $PtdIns(3,4,5)P_3$) is synthesized by phosphorylation of PIP_2 by the enzyme ***phosphatidylinositol-3-kinase*** (PI3K). It remains bound in the inner leaflet of the cytoplasmic membrane, where it activates phosphatidylinositol-dependent kinase 1 (PDK1), which phosphorylates (and thus activates) **protein kinase B**. Protein kinase B (PKB, also called AKT) phosphorylates proteins regulating proliferation, cell cycle a apoptosis.



Diacylglycerol and inositol-1,4,5-trisphosphate

The signal molecule binds to the receptor, and this leads to the activation of the enzyme phospholipase C, which splits phosphatidylinositol-4,5-bisphosphate into 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3, also $Ins(1,4,5)P_3$). Both are second messengers. IP_3 diffuses through the cytosol, binds to a calcium channel in the endoplasmic reticulum and thereby opens it. Calcium cations are released from the Endoplasmic reticulum and increase the level of Ca^{2+} in the cytosol. DAG remains embedded in the cytoplasmic membrane. DAG and calcium ions together activate **protein kinase C**, which subsequently phosphorylates proteins associated with cytoskeleton and thereby affects contraction, migration and secretion in cells.



Calcium cations

Ca^{2+} is a more common second messenger than cAMP. Increasing the concentration of Ca^{2+} causes muscle cell contraction or the secretion of certain substances. Cells still contain some amount of Ca^{2+}, but the extracellular level is far higher than the intracellular level. The intracellular level is increased by the opening of calcium channels in the endoplasmic reticulum, which is caused by the binding of IP_3 to these channels. Calcium cations could be considered third messengers. Calcium cations activate other proteins either directly or with the help of **calmodulin**, a Ca^{2+}-binding protein. Calmodulin with bound calcium ions activates CaM-kinases, which phosphorylate other proteins.

Links

Related articles

- Hormone
- G-protein
- Insulin
- Cytokines
- Growth factor
- Second messengers

Reference

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2. MATOUŠ, Bohuslav. *Základy lékařské chemie a biochemie.* 1. edition. Galén, 2010. 540 pp. ISBN 978-80-7262-702-8.

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