Control of metabolism
Mechanism of hormone and neurotransmitter action
There are three formal levels, in which the control of metabolism is achieved:

– Regulation of metabolic events within particular compartment (cellular organelle) that depends only on interactions between molecules in the compartment;
– regulations that occur within complete cells without any regard to extracellular signals, in which proteosynthesis and transport across membranes that separate individual compartments have the important roles have;
– regulations that are consequences of communication between cells in particular tissues, organs, or the whole organism, depending on extracellular signals – neurotransmitters, hormones, cytokines, and other signal molecules.

Numerous metabolic pathways are controlled usually in only one or few check-points (rate-limiting steps) by more than one different mechanisms.

These formal levels of metabolism control mostly overlap.
Some factors important in control of metabolism:

- Primarily, the **equipment of cells with enzymes and other proteins (the proteome)**, which is determined by the expression of genes in the given cell type within the given time period.

- **Specific receptors**, which enable recognition of extracellular signal molecules as well as reactions of the cell or body to changes in the environment.

- The **existence of multiple enzyme forms** (isoenzymes) allows to control particular reaction types by different mechanisms in various compartments, various tissues, or in various time periods.

- **Accessibility of nutrients** and other essential substances, on which the **energetic state of the cell** depends.
Three major mechanisms that provide control of metabolism

1. Regulation of the amount of enzymes (number of enzyme molecules) present in the cell.

2. Regulation of enzyme activity or activity of regulatory proteins, on which the activities of enzymes depend.

3. Regulation of transport across membranes that separate intracellular and extracellular spaces as well as individual cellular compartments.
Regulation of proteosynthesis:

The expression of some genes occurs at a nearly constant rate (synthesis of constitutive enzymes). Numerous genes are expressed in response to specific regulatory signals, expression of some other may be silenced. The enzymes controlled in this way are adaptable enzymes (mostly inducible, see chapter Regulation of gene expression).

Regulation of proteosynthesis may occur at the level of gene amplification, transcription, posttranscriptional hnRNA processing (alternate mRNA splicing), export of mRNA from nucleus, degradation of mRNA, translation, and posttranslational modification.

In eukaryotes, expression of genes can be induced by binding of signal molecules on specific membrane receptors (e.g. growth factors, cytokines, and insulin), or by interactions of hydrophobic signal molecules (steroid hormones, iodothyronines, retinoates) with specific intracellular receptors.
1 Regulation of the amount of enzymes

- **Regulation of enzyme degradation:**

  Rates of degradation of specific enzymes are selectively regulated, namely of those that catalyze the rate-limiting steps in biochemical pathways or represent important metabolic control points. Those enzymes are mostly short-lived proteins (biological half-lives from several minutes to few hours) and their degradation is provided by cytosolic ubiquitin system, or by other systems not yet known.

  The susceptibility of an enzyme to proteolytic degradation depends upon its conformation that may be altered by the presence or absence of substrates, coenzymes, and metal ions.

  **Long-lived proteins**, under physiological conditions, are degraded at nearly constant rates, mostly nonselectively.

  **Nutritional deprivation** (starving) increases selectively the degradation rates of enzymes that can be missed and are not necessary for survival of the cell.
Regulation of enzyme activity

is a more rapid type of control than the control of enzyme synthesis. The enzyme activities can be changed effectively in several ways:

– activation of proenzymes by partial proteolysis of the proenzyme,

– allosteric control and cooperative effects of enzymes that consist of several identical subunits,

– control arising from interactions with regulatory proteins (e.g. activation of enzymes by releasing of inhibitory subunits or another regulatory protein),

– control by reversible covalent modification of enzymes or of regulatory proteins; the most important example of this is reversible phosphorylation, catalyzed by protein kinases and controlled by extracellular signals.
Active enzymes are formed from proenzymes molecules by irreversible splitting of certain part(s) in their polypeptide chain. This principle of activation is frequent among proteinases, because it prevents against unwanted breakdown of proteins.

Examples:
Extracellular – "big“ proteinases of the gastrointestinal tract (pepsin, chymotrypsin, trypsin, etc.),
– proteinases in the blood clotting cascade (coagulation factors IX, X, XI, and thrombin);
intracellular proteinases – activation of caspases that initiate apoptosis).
2 Regulation of enzyme activity

– Allosteric regulation of activity and cooperative effects

Regulatory enzymes are frequently **oligomers** that consist of several identical subunits (protomers). Their saturation curves usually deviate from hyperbolic (Michaelis) shape, they are **sigmoid**.

**Cooperative effect** – In these oligomeric enzymes (and also in some non-catalysts, e.g. haemoglobin) the binding of **substrates** (or $O_2$ to haemoglobin, resp.) to one of the **active sites** can affect the affinity of active sites for substrates in the other subunits. The effect becomes **positively cooperative**, when it facilitates, due to induced changes in conformation, substrate binding to the other subunits and so activates the enzyme.

**Allosteric effectors** are molecules that are allosteric to the substrate (having **structures distinct from the substrate**) and can bind reversibly to **specific sites other than the enzymes´ active sites** (to the allosteric sites). The induced change in conformation results either in higher activity of the enzymes or in inhibition.
### Regulation of allosteric enzymes – examples:

<table>
<thead>
<tr>
<th>Allosteric enzyme</th>
<th>Cooperative effect of the substrate</th>
<th>Allosteric activator</th>
<th>Allosteric inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen synthase</td>
<td>–</td>
<td>Glc-6-P</td>
<td>-</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>–</td>
<td>Glc-1-P, AMP</td>
<td>Glc-6-P</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>Fru-6-P</td>
<td>Fru-2,6-P₂, ADP</td>
<td>citrate, ATP</td>
</tr>
<tr>
<td>Fru-1,6-bisphosphatase</td>
<td>Fru-1,6-P₂</td>
<td>phosphoenolpyruvate</td>
<td>Fru-2,6-P₂</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>phosphoenolpyruvate</td>
<td>Fru-1,6-P₂</td>
<td>alanine</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td>–</td>
<td>–</td>
<td>acetyl-CoA, ATP, NADH</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>–</td>
<td>ADP</td>
<td>ATP, NADH</td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>–</td>
<td>acetyl-CoA</td>
<td>citrate</td>
</tr>
</tbody>
</table>
2 Regulation of enzyme activity

– Control of enzyme activity by regulatory protein

Examples: Protein kinase A forms inactive tetramers $C_2R_2$. If two regulatory subunits R bind four molecules cAMP, two catalytically active subunits C are released. The decrease in cAMP concentration supports interactions between C and R subunits, the inactive tetramer is restored.

Phosphoprotein phosphatase 1 has a regulatory subunit, which keeps up active complex of glycogen with the catalytic subunit. If the regulatory unit is phosphorylated by PKA, it releases the catalytic subunit (exhibiting low activity) that is then fully inactivated by binding with an similarly phosphorylated protein inhibitor. If it is phosphorylated at another site by insulin-dependent PK, the phosphatase activity of the complex of glycogen and the catalytic subunit will increase.

Proteinases often occur in the inactive forms, bound reversibly to the more or less specific proteins (proteinase inhibitors). Plasma proteinase thrombin is inactivated by binding to antithrombin, intracellular Ser- or Cys-proteinases are inhibited by various types of serpins and cystatins.
2 Regulation of enzyme activity

- **Reversible covalent modification of proteins:**
  - **phosphorylation** of proteins catalyzed by *protein kinases* (PK); phosphate ester originates by the transfer of $\gamma$-phosphate from ATP, dephosphorylation (hydrolysis) is catalyzed by *phosphoprotein phosphatases*;
  - **acetylation** (e.g., of histones in nucleosomes), through transfer of acetyl from acetyl-CoA;
  - **ADP-ribosylation** (e.g. $G_\alpha_S$, EF-2, RNA polymerases), transfer of ADP-ribosyl from NAD$^+$, nicotinamide is released;
  - **myristoylation**, **farnesylation** (prenylation), and many other.

$\gamma$-**Carboxylation** of glutamyl residues side chains (prothrombin and other factors in the blood-clotting cascade, osteocalcin, etc.) is obviously **irreversible**, but it is important in formation of binding centres for Ca$^{2+}$ ions, essential for the biological activity of the protein.
Reversible phosphorylation of proteins is an intracellular reaction. ATP is the donor of phosphate.

Phosphorylation is catalyzed by highly specific protein kinases (PK).

Protein kinases are the largest family of homologous enzymes known – there are more than 550 human types of protein kinases.

Proteins are phosphorylated either on serine or threonine residues (alcoholic groups), or on residues of tyrosine (phenolic hydroxyl), at specific positions within the polypeptide chains.

Activation of various protein kinases is specific – e.g. cAMP, cGMP, Ca\(^{2+}\)-calmodulin complex, etc. (see next table).

The signal that activates protein kinases is amplified (activation of one enzyme molecule results in phosphorylation of numerous protein molecules).

Dephosphorylation of phosphoproteins (hydrolysis of the ester bond) is catalyzed by phosphoprotein phosphatases.
2 Regulation of enzyme activity

**Examples of protein kinases (PKs):**

<table>
<thead>
<tr>
<th>Phosphorylation of Ser/Thr residues</th>
<th>Activated by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinases <strong>A</strong></td>
<td>cAMP</td>
</tr>
<tr>
<td>Protein kinases <strong>G</strong></td>
<td>cGMP</td>
</tr>
<tr>
<td>Protein kinases <strong>C</strong></td>
<td>diacylglycerol (and Ca(^{2+}))</td>
</tr>
<tr>
<td><strong>AMP</strong>-dependent PK</td>
<td>AMP</td>
</tr>
<tr>
<td><strong>Ca(^{2+}/CaM)</strong>-dependent PKs</td>
<td>Ca(^{2+}) or Ca(^{2+})-calmodulin</td>
</tr>
<tr>
<td><strong>PIP(_3)</strong>-dependent PK-1</td>
<td>phosphoinositol 3,4,5-trisphosphate</td>
</tr>
<tr>
<td><strong>Mitogen</strong>-activated PKs (MAP, MAPKK)</td>
<td>growth factors, cellular stress</td>
</tr>
<tr>
<td><strong>Cyclin</strong>-dependent PK</td>
<td>cyclins (regulatory proteins)</td>
</tr>
</tbody>
</table>

**Phosphorylation of tyrosine residues (tyrosine kinases)**

- **receptor types** – e.g., insulin receptor or receptors of some growth factors (IGF1,2, epidermal growth factor)
- **intracellular, non-receptor types** (e.g., Janus kinases) activated by membrane receptors of growth hormone, prolactin, erythropoietin, cytokines.
### Examples of regulation by reversible phosphorylation:

<table>
<thead>
<tr>
<th><strong>Activated by phosphorylation</strong></th>
<th><strong>Inhibited by phosphorylation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>glycogen phosphorylase-b-kinase</td>
<td>glycogen synthase</td>
</tr>
<tr>
<td>glycogen phosphorylase</td>
<td>(glycogen synthesis)</td>
</tr>
<tr>
<td>(glycogenolysis)</td>
<td>fructose 6-phosphate 2-kinase</td>
</tr>
<tr>
<td>fructose 2,6-bisphosphatase</td>
<td>pyruvate dehydrogenase</td>
</tr>
<tr>
<td>(gluconeogenesis)</td>
<td>(glycolysis)</td>
</tr>
<tr>
<td></td>
<td>acetyl-CoA carboxylase</td>
</tr>
<tr>
<td></td>
<td>(fatty acid synthesis)</td>
</tr>
<tr>
<td></td>
<td>HMG-CoA reductase</td>
</tr>
<tr>
<td></td>
<td>(cholesterol synthesis)</td>
</tr>
</tbody>
</table>
3 Regulation of the transport across membranes

Examples:

— Insulin stimulates glycolysis, because it also promotes the uptake of glucose by muscle and adipose tissue. Binding of insulin to its receptor leads to a rapid increase in the number of GLUT4 transporters in the plasma membrane of rhabdomyocytes and adipocytes.

— The fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active. Malonyl-CoA (present in cytosol when there is an abundant supply of nutrients to the cell) inhibits carnitine acyltransferase I, thus preventing access of fatty acyl-CoAs to the mitochondrial matrix and the enzymes that catalyze their oxidation. On the contrary, fatty acyl-CoAs (present in cytosol at a high level in fasting) inhibit the mitochondrial tricarboxylate transporter, thus preventing activation of acetyl-CoA carboxylase by outflow of citrate from mitochondrial matrix.
Mechanism of hormone and neurotransmitter action

Signal molecule types in neurohumoral regulations:

**HORMONES** secreted by endocrine glands, by dispersed glandular cells (eicosanoids by many other cellular types);

**NEUROHORMONES** secreted by neurons into the blood circulation;

**NEUROTRANSMITTERS** secreted by neurons at nerve endings;

**CYTOKINES** secreted by immunocompetent cells;

**GROWTH FACTORS** secreted by various types of cells.

Signal molecules can be also classified as
- **endocrine** - carried by the blood, may act in the whole body,
- **paracrine** - act within short distances of the site of their production,
- **autocrine** - act on the cells that produce them.
Hierarchical arrangement and signal amplification of some regulatory processes

Example:

Neurons of brain cortex: NEUROTRANSMITTERS at most nanograms

Specific neurons in the hypothalamus: CORTICOLIBERIN micrograms

Cells in the anterior lobe of the pituitary: CORTICOTROPIN hundreds of micrograms

Cells of the adrenal cortex: CORTISOL tens of milligrams

CELLS IN THE TARGET TISSUES
Factors influencing the biological effects of hormones:

- HORMONE SYNTHESIS
- HORMONE STORAGE
- SECRETION
- TRANSPORT
- TARGET CELL RECEPTOR SIGNAL-TRANSDUCTION PATHWAY
- BIOLOGICAL EFFECT

CONTROL OF GENE EXPRESSION
SECRETION STIMULUS
- specific
  - e.g. liberin, glandotropin
- non-specific
  - e.g. Ca^{2+}, H^{+}

FEED-BACK CONTROL
Metabolism INACTIVATION
EXCRETION

The hormone concentrations in blood plasma need not correlate with the biological effects!
TRANSDUCTION OF EXTRACELLULAR SIGNALS

How cells receive, process, and respond to information from the environment?

The size and polarity of a signal molecule is decisive.

– **Proteins and small polar signal molecules** (amino acids, peptides, biogenic amines, eicosanoids) don't penetrate across plasma membranes. They bind onto specific [membrane receptors](#) (integral membrane proteins).

  Binding of the ligand to the receptor results in a conformational change of the intracellular domain, which either generates an increase of intracellular concentration of a small **secondary signal molecule** (the second messenger), or directly activates a protein kinase.

– **Nonpolar signal molecules** (steroids, iodothyronines, retinoates) diffuse through the plasma membranes of all cells and bind to specific proteins - [intracellular receptors](#).

  Complexes hormone-receptor then enter the nuclei, binds to a specific region of DNA (hormone response element, HRE), and activate (or repress) gene transcription.
Membrane and intracellular receptors

**Polar** signal molecule

**Membrane receptor**

Signal transduction

Amplification

Biological response

(prompt effect)

**Nonpolar** signal molecule

**Membrane receptor**

**Intracellular receptor**

Interaction of the complex hormone-receptor with DNA

Biological response

(the effect is slow, either early or late)
Main types of membrane receptors

Receptors – **ion-channels** (ROC, ligand gated ionophores) serve exclusively as receptors for neurotransmitters (see lecture 7).

**Receptors activating G-proteins** (heterotrimeric G-proteins), the result of specific ligand binding is mostly
- stimulation or inhibition of **adenylate cyclase**,  
- stimulation of **phospholipase C**,  
- stimulation of **phosphodiesterase**.

**Receptors exhibiting intrinsic catalytic activity**
- **guanulate cyclase activity** – receptors for natriuretic peptides,  
- **tyrosine kinase activity**  
  - insulin receptor, receptors for insulin-li growth factors (IGF1,2),  
  - dimerizing receptor for epidermal growth factor (EGF).

**Receptors cooperating with non-receptor tyrosine kinases**
(e.g., Janus kinase, JAK) – receptors for somatotropin (growth hormone), prolactin, erythropoietin, interferons, interleukins and other cytokines.
Family of heterotrimeric G-protein-coupled receptors

All receptors of this type exhibit **common structural features:**

*Extracellular parts* (the N-end and hydrophilic loops) are slightly glycosylated; α-helical segments IV, VI, and VII form a "pocket", the specific **binding site for the agonist**. There are also accessory binding sites for antagonists.

**Seven α-helical segments** span the membrane and are connected by intra- and extracellular hydrophilic and more divergent loops.

*Intracellular domains* represent the binding site for the **specific G-protein type**.
G-proteins

are **GTP-** and/or **GDP-binding proteins**, mostly freely membrane-bound (they can move along the inner surface of the plasma membrane). G-proteins participate in various types of the second messenger production. All types of those G-proteins have a similar structure and mechanism of activation.

**Heterotrimers** consist of subunits \( \alpha \), \( \beta \), and \( \gamma \).

\( \text{G} \beta \) and \( \text{G} \gamma \) subunits are hydrophobic and **nonspecific**, \( \text{G} \alpha \) subunit is the largest, hydrophilic, it binds GTP or GDP, and is **specific** for particular mechanism of second messenger production.

More than 20 different \( \alpha \) subunits have been identified. Examples – see table (picture number 26).
The cycle of G-proteins activation

Complex receptor-specific ligand

Trimer $G\alpha$-GDP, $G\beta$, $G\gamma$

Dimer $G\beta$, $G\gamma$

Inactive subunit $G\alpha$-GDP

Interaction with the target protein

PRODUCTION OF THE SECOND MESSENGER

Activated subunit $G\alpha$-GTP

$GTP$

GDP

$G\alpha$-GDP, $G\beta$, $G\gamma$

$Pi$

PRODUCTION OF THE SECOND MESSENGER
### Selected types of G protein α-subunits

<table>
<thead>
<tr>
<th>G_α subunit type</th>
<th>Examples of activating receptors</th>
<th>Effect of activated G_α on the target protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>G_α is (s for stimulatory)</td>
<td>glucagon, parathyrin, β-adrenergic</td>
<td>stimulation of adenylate cyclase</td>
</tr>
<tr>
<td>G_α i (i for inhibitory)</td>
<td>somatostatin, α_2-adrenergic</td>
<td>inhibition of adenylate cyclase</td>
</tr>
<tr>
<td>G_α q (activating the PI cascade)</td>
<td>vasopressin V_1, endothelin ET_{A,B}, acetylcholine M_1, α_1-adrenergic</td>
<td>stimulation of phospholipase C</td>
</tr>
<tr>
<td>G_α t (t for transducin)</td>
<td>rhodopsin</td>
<td>stimulation of cGMP phosphodiesterase</td>
</tr>
</tbody>
</table>
Hormone receptors that activate G\textsubscript{s} or G\textsubscript{i} proteins stimulates or inhibit adenylate cyclase

*Adenylate cyclase*, a membrane-bound enzyme, catalyzes the reaction ATP $\rightarrow$ cAMP + PP\textsubscript{i}; the second messenger is cyclic AMP.
Receptors that activate Gq protein stimulate phospholipase C and start the phosphatidylinositol cascade

*Phospholipase C* catalyzes hydrolysis of phosphodiester bond in phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate:

![Chemical structures](image)

**Both** reaction products are the second messengers:
Inositol 1,4,5-trisphosphate opens the Ca^{2+} channel in ER membrane, diacylglycerol activates protein kinase C.
Phosphatidylinositol cascade

specific ligand

receptor

activation of proteinkinase C

proteinkinase C

increase of $[\text{Ca}^{2+}]$

in cytoplasm

Endoplasmic reticulum

$\text{IP}_3$ receptors in the membranes of ER act as ligand gated channels for $\text{Ca}^{2+}$ ions
**Receptors having intrinsic catalytic activities**

**Insulin receptors has an intrinsic tyrosine kinase activity** of the intracellular domains of β subunits.

Binding of insulin to its specific receptor stimulates autophosphorylation of β subunits and **phosphorylation of IRS 1-4** (insulin receptor substrates 1-4).

- Activation and docking of PI-3-kinase on membrane
- Activation of phosphoprotein phosphatase-1
- Activation of small G-protein Ras
Insulin receptor substrates 1-4 are **adaptor proteins**. If phosphorylated by the insulin-receptor complex, they bind to other proteins that are activated in this way.

Among others,

- the lipid kinase **PIP₂ 3-kinase** is activated. The product PIP₃ initiates activation of the kinase **PDK-1** (PIP₃-dependent kinase) which, in turn, activates protein kinase **PK B**. The consequence is exposition of transporters GLUT4 into membranes of skeletal muscles and adipocytes.

- Regulatory subunit of **phosphoprotein phosphatase-1** is activated resulting in activation of its phosphatase activity which **dephosphorylates** both glycogen synthase and phosphorylase.

- Phosphorylation of IRS also results in docking of proteins Grb2 and SoS and activation of small **G-protein Ras** which triggers, through binding onto protein **kinase Raf**, the cascade of phosphorylations called the **Ras signalling pathway** (mitogen-activated protein kinases, MAPKs) important in the regulation of proliferation and differentiation of several cell types.
Dimerizing receptor for EGF (epidermal growth factor) containing an intrinsic tyrosine kinase activity

Autophosphorylation of the receptor enables linking of proteins Grb2 and SoS which bind and so activate the Ras signalling pathway. The small G-protein Ras (Ras-GDP) after an exchange of GDP for GTP activates the serine protein kinase Raf and initiates the phosphorylation cascade catalyzed by protein kinases MAPKs (mitogen-activated PKs) and ERKs (extracellular signal-regulated PKs).

The consequence is phosphorylation of transcription factors and regulation of gene expression.
Receptors activating non-receptor tyrosine kinases

Dimerizing receptors activating tyrosine kinases JAK (Janus kinases) – e.g., receptors for prolactin, growth hormone, erythropoietin, interferon, various interleukins and other cytokines.

Upon ligand binding, these receptors dimerize and interact with a cytosolic tyrosine kinase **JAK** which is autophosphorylated and phosphorylates the receptor on tyrosine residues. The **STAT proteins** (signal transducers and activators of transcription) associate with the receptor and are phosphorylated by JAK. STAT phosphates dimerize, translocate to the nucleus, bind to specific DNA elements and regulate transcription. In a similar way, phosphorylated receptors activate **MAP kinase cascade**.
Intracellular receptors of steroid hormones (and calcitriols), iodothyronines, and retinoates

The general features of the function of all these receptors are very similar. The hormone-receptor complexes binds to specific regions of DNA (called hormone response elements, HRE) and activate or inactivate transcription of specific genes.

*hydrophobic hormone molecule* (cortisol) diffuses into the cell

*active complex receptor-ligand* (monomer), hsp 90 and other proteins are released

inactive receptor for glucocorticoids (GR) forms in the cytoplasm complex with hsp 90 dimer and other proteins

active complexes form *dimers* and are translocated into the nucleus through nuclear pores
Glucocorticoid receptor (GR) – function

Active complex cortisol-receptor binds onto DNA at the specific sequence GRE (glucocorticoid response element, quite generally HRE – hormone response element), after the coactivators and specific hormone response element-binding proteins (HREB-proteins) has been attached. So the complex acquires the ability to act as enhancer that supports initiation of transcription on the promoter.
Initiation of transcription by cortisol

Active complex cortisol-receptor binds onto DNA at the specific sequence GRE (glucocorticoid response element, one of the HRE – hormone response elements).

The coactivator and specific hormone response element-binding proteins (GREB-proteins) are also attached. This complex acquires the ability to act as enhancer that supports initiation of transcription on the promoter by means of mediator proteins.

GR dimer – intracellular glucocorticoid receptor (dimer)
GRE – glucocorticoid response element
GREB protein – GRE binding protein (a specific transcription factor)