Regulation of metabolic pathways at the cell level

Subchapter content

General principles of regulation of metabolic pathways at the cell level

The activity of metabolic pathways must be constantly changed by the organism. It is regulated both according to the immediate needs and possibilities of a particular cell, and according to the needs of the organism as a whole.

At the level of the organism, regulation is mediated by signals that come to the cells from the outside. They are transferred across the cell membrane and inside they connect with the regulatory events of the cell itself. Regulations tend to have a number of steps that fall into **cascades** . It often ends **by changing the activity of the enzyme** , which thus acts as an **effector** of regulation. Usually only one enzyme of the metabolic pathway is subject to regulation, the so-called **key** or **regulatory enzyme** . Key enzymes usually catalyze the slowest reactions of metabolic pathways.

The speed of a certain **metabolic pathway** as a whole is determined by its slowest step. As we have already stated, it is this step that is usually subject to regulation. Both the **number of key enzyme molecules** and their activity (i.e. **catalytic efficiency**) can change. Key enzymes often catalyze **practically irreversible** , e.g. strongly exergonic reactions.

Events that change the speed of metabolic pathways can be divided into three groups:

1. **Regulation** that uses compartmentalization of metabolic events
2. **Enzyme concentration control**
3. **Modulation of activity** (catalytic efficiency) of enzyme molecules

Compartmentation of metabolic events

A eukaryotic cell is divided into several **compartments by** semipermeable membranes . They differ from each other in, for example, enzymatic equipment or membrane transporters. The pH values are also different - enzymes often have different pH optima . If there was only one space in the cell, some of the enzymes would probably not be functional or the catalysis mediated by them would not be efficient enough.

See Compartmentation of Metabolic Pathways (FBLT) for more detailed information .

Overview of metabolic pathways according to the compartments in which they take place

Cell partition	Metabolic pathways
Cytoplasm	Carbohydrate metabolism: glycolysis, part of gluconeogenesis , glycogenolysis and glycogen synthesis, pentose cycle Fatty acid metabolism: synthesis of fatty acids Amino acid metabolism: synthesis of non-essential Fatty acids, some transaminations Other pathways: part of the heme and urea synthesis pathways , purine and pyrimidine metabolism.
Mitochondria	Carbohydrate metabolism: pyruvate dehydrogenase complex, beginning of gluconeogenesis (conversion of pyruvate to oxaloacetate) Fatty acid metabolism: β-oxidation of fatty acids, synthesis of ketone bodies (only liver cells), degradation of ketone bodies (only extrahepatic tissues) Metabolism of amino acids: oxidative deamination of glutamate, some transaminations Other pathways: Krebs cycle , respiratory chain, and oxidative phosphorylation (on the inner mitochondrial membrane), part of heme and urea synthesis.
Smooth endoplasmic reticulum	Synthesis of triacylglycerols and phospholipids Elongation and desaturation of fatty acids Part of steroid synthesis Biotransformation of xenobiotics Conversion of glucose-6-phosphate to glucose (in tissues where glucose-6-phosphatase occurs)
Rough endoplasmic reticulum	Proteosynthesis (translation of mRNA) Post-translational modifications (oxidation, cleavage, methylation, phosphorylation, glycosylation)
Golgi apparatus	Post-translational modification of proteins (glycosylation, ...) Sorting of proteins and formation of secretory vesicles
Lysosomes	Hydrolytic cleavage of proteins, carbohydrates, lipids and nucleic acids
Peroxisomes	Degradation of MK with a long chain (from 20 carbons)
Nucleus	DNA replication and transcription RNA synthesis
Nucleolus	RNA editing Synthesis of ribosomes
Ribosomes	Protein synthesis

We also observe a different distribution of substrates and products in different compartments of the cell. Even some coenzymes cannot pass freely between compartments, e.g. molecules of NADH or coenzyme A do not pass through the inner mitochondrial membrane. Many enzymes need a suitable coenzyme for their catalytic function. By changing the concentration of a coenzyme in a certain compartment, a certain metabolic pathway can be turned on or off. Compartmentation also facilitates the **regulation of conflicting events** .

E.g. the synthesis of fatty acids takes place in the cytoplasm, while their degradation takes place in the mitochondria. The speed of reactions depends on

- supply of substrates, or cosubstrates (coenzymes),
  - from the previous steps of the metabolic pathway,
  - by transport from other compartments,
- pumping out products
  - further steps of the metabolic pathway,
  - by transport to other compartments

E.g. The Krebs cycle would stop if the NADH it forms is not used up in the respiratory chain. The respiratory chain reoxidizes NADH to NAD + , which re-enters the Krebs cycle as a coenzyme.

Sometimes an excess of citrate is produced in the mitochondria. The latter can be transported into the cytoplasm, where it acts as a regulatory molecule.

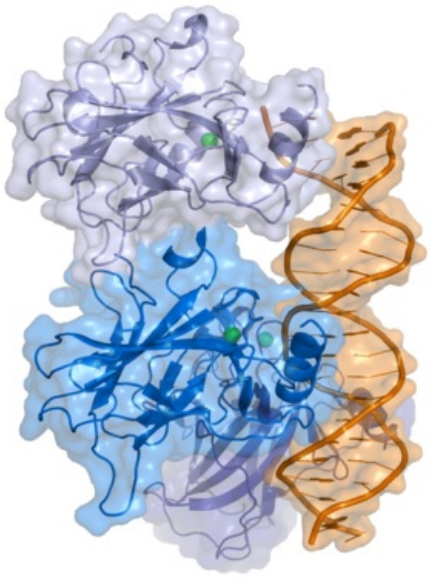
Reactions that directly follow each other in metabolism often take place on enzymes that are in close proximity. Examples can be the reactions of the already mentioned Krebs cycle or the respiratory chain. The grouping of reactions into one compartment increases the speed of metabolic pathways, as the product of one reaction accumulates directly in the place where it serves as a substrate for subsequent reactions.

Compartmentation allows sensitive and targeted control of metabolic pathways that take place in one place without affecting processes in another part of the cell.

Compartmentation places increased demands on the energy consumption of the cell. Transport of substances across membranes often goes against the concentration gradient and must use ATP-dependent transporters .

Change in enzyme concentration

The amount of an enzyme in a cell is changed by increasing or decreasing **the expression of the gene** that codes for the enzyme. A regulatory protein that affects transcription is called a transcription factor - inducer or repressor. The action of regulatory proteins is usually reversible. Transcription factors are dependent on certain molecules (e.g. hormones) that act as their ligands. Regulation by this route takes longer (hours to days) than regulation of the activity of an already formed enzyme (seconds, minutes).



The p53 transcription factor complexed with DNA

Gene expression is generally influenced by four different mechanisms.

- A ligand is attached to the repressor, which is bound to the DNA. The repressor-ligand complex is released, allowing gene transcription.
- The ligand binds to the free repressor. The repressor-ligand complex binds to DNA and disables gene transcription.
- A ligand is attached to the inducer, which is bound to the DNA. The inducer-ligand complex is released, preventing gene transcription.
- The ligand binds to the free inducer. The inducer-ligand complex binds to the DNA and thus enables gene transcription.

Induction of enzyme formation can increase its quantity several times.

For example, glucocorticoids induce gluconeogenesis enzymes .

Conversely, **repression** can significantly reduce enzyme production.

For example, heme reduces the formation of delta-aminolevulate synthase.

Through these processes, cells adapt to the changing internal environment.

Modulation of the activity of an already existing enzyme

Covalent modification of the enzyme molecule

Formation of active enzymes from inactive precursors

Many enzymes are formed in an inactive form (so-called **proenzymes** or **zymogens**). Partial proteolysis of the enzyme changes the molecule into an active form and increases the concentration of the active enzyme. Eliminating the enzymes activated in this way arranges their proteolytic degradation. This process is typical, for example, of digestive enzymes or some factors of the coagulation cascade .

Interconversion of enzymes

Enzyme interconversion is the rapid switching between the active and inactive form of an enzyme molecule by another enzyme. The best-known example is the reversible ATP-dependent phosphorylation and dephosphorylation of the hydroxyl groups of serine , threonine or tyrosine amino acids forming the enzyme chains.

Some enzymes are **activated** by phosphorylation (e.g. glycogen phosphorylase), others are **inhibited** by phosphorylation (e.g. glycogen synthase). Phosphorylation is catalyzed by enzymes belonging to **protein kinases** (phosphotransferases), dephosphorylation is ensured by **protein phosphatases**. This method of regulation of enzyme activity is mainly applied when transmitting a signal from membrane receptors to the inside of cells, for example when responding to a hormonal signal (the connection between the signal coming to the cells from the outside and the subsequent influencing of events inside them).

Interventions that affect enzyme kinetics

Effect of concentration of substrates and products, KM values , pH, temperature, etc.

- **Availability of substrates**

  The concentration of regulatory enzymes of metabolic pathways is very low in the cell. Likewise, the substrate concentration is much lower than the value of the Michaelis constant (K M coincides with the substrate

concentration at which the rate of the enzyme-catalyzed reaction reaches half the maximum rate). Even a slight change in the concentration of the substrate will change the speed of its transformation.

The change in substrate concentration takes place through increased intake, synthesis or transport of the substrate to the place where we metabolize it.

- **The influence of the Michaelis constant KM**

  Enzymes have a certain substrate specificity. If an enzyme converts several different substrates, it has a different affinity for each substrate. If, for example, two different enzymes can convert the same substrate, each of them has a different affinity for the given substrate. The higher the affinity to the substrate (the lower the K M for the enzyme-substrate pair), the lower the concentration of the substrate in the vicinity of its active center, which is necessary for the reaction to take place, is enough for the enzyme.

- **Removal of Products**

  If the reaction product is used immediately, it does not accumulate and the reaction continues in the direction of its further formation. If the unused product begins to accumulate, it often serves as an inhibitor of the reaction or sequence of reactions leading to its formation. The following mechanisms prevent this in metabolism:

  1. removing the product of one reaction by a subsequent reaction (principle of metabolic pathways),
  2. utilization of the product of one metabolic pathway in another metabolic pathway,
  3. transport of the product to another cell compartment.

  All these processes will speed up the course of the given reaction.

- **Effect of pH**

  A change in pH can also affect enzyme activity by changing the dissociation of functional groups in the **active center** of the enzyme (electrostatic interactions during substrate binding) as well as in **the entire enzyme molecule** (changing the biologically active conformation of the enzyme in a less active conformation – e.g. access to the active center).

Effect of the presence of activity modulators (activators or inhibitors)

The activity of a regulatory enzyme can be affected by the direct binding of some substance (ligand or effector or modulator) to its protein molecule. A positive effector serves as an activator of the enzyme, while a negative effector is its inhibitor.

- The accumulation of the final product (or intermediate) of a given metabolic pathway often leads to the inhibition of the regulatory enzyme of the given pathway, to which the product binds - so-called **feedback regulation .**
- The (inter)product of one metabolic pathway can influence (activate or inhibit) the activity of the regulatory enzyme of another, somehow related metabolic pathway – the so-called **cross-regulation .**
- An intermediate product of a metabolic pathway can influence the activity of a subsequent enzyme of a given metabolic pathway – the so-called **step-forward regulation .**

Modulators

Modulators can bind to the enzyme either directly in the active center (competitive inhibition), or bind to another, so-called **allosteric site (**allosteric regulation). Natural activity modulators bind to the enzyme non-covalently, using only weak non-binding interactions .

Isosteric modulation of enzyme activity

Isosteric modulation of enzyme activity refers to **simple enzymes** that exhibit a hyperbolic dependence between reaction rate and substrate concentration. Their activity is mainly affected by changes in substrate concentration, reduction or increase in enzyme synthesis. Furthermore, the activity of enzymes is affected by inhibitors that bind directly to the active center **instead of the substrate** (competitive inhibition). A competitive inhibitor can be one of the products of the metabolic pathway (feedback inhibition).

Allosteric regulation

Allosteric regulation occurs in **multi-subunit enzymes** (most regulatory enzymes of metabolic pathways). These enzymes show a **sigmoidal dependence** of the reaction rate on the substrate concentration. Allosteric modulators of activity bind outside the **active** site to other sites on the enzyme molecule. The binding of the modulator changes the conformation of the molecule, which can be manifested **by a change in the affinity** of the enzyme to the substrate (i.e., a decrease or increase in the value of KM).

The concentration of the active enzyme can also be reduced (part of the enzyme molecules is inactivated), thereby causing a change in the value of the maximum rate of the enzyme-catalyzed reaction. By binding the activator, the less active so-called **T-form of the enzyme** ("tension") changes to the more active **R-form** ("relaxed"). The binding of allosteric activators can thus increase the number of enzyme molecules in the R-form.

Substrates and effectors generally only affect the balance between the T and R conformations—the two conformations coexist in varying proportions. The final amount of active forms of the enzyme depends on the total effect of various activators and inhibitors binding to the enzyme, i.e. it depends on their **mutual ratio** in the cell. With an increase in K M , the reaction can be completely eliminated under physiological conditions, since the physiological concentration of the substrate lies in the region where the enzyme is practically ineffective. It is a very fine and continuous regulation of the reaction rate based on the connection of various metabolic pathways.

Regulation of individual metabolic pathways

More detailed information can be found on the Regulation of Individual Metabolic Pathways (FBLT) page .