

Chapter 2

Biochemical Fundamentals

Tiina Liiving, Syed M. Baker,
and Björn H. Junker

Abstract Living organisms are among the most complex phenomena in our world. To describe, model, and simulate living organisms or at least parts thereof, formal descriptions such as Petri nets are needed. As the focus of this book is the use of Petri net theory in biology, the readership will be very diverse. Thus, this chapter is meant to provide a general introduction to biology, especially those areas that will be modeled with the use of Petri net approaches throughout this book. The experienced biochemist might want to skip this chapter, but for computer scientists and readers from similar fields this chapter contains important fundamentals.

2.1 Cell Biology

A cell is the smallest highly organized basic life form from which all living organisms are built. Thus, a cell might be called the “building block of life”. The word *cell* comes from the Latin word *cellula* which means *small room*. Each cell is self-contained and self-maintaining and has its own set of instructions for carrying out all of its activities.

Cells can be categorized in two main types, eukaryotic and prokaryotic, which principally differ from each other by having or not having a membrane enclosed nucleus. The names of the cell types indicate this property as in Greek *karyose* means *kernel*, *pro* means *before*, and *eu* means *true* or *good*. Eukaryotic cells have

T. Liiving · S.M. Baker · B.H. Junker (✉)
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3,
06466 Gatersleben, Germany
e-mail: junker@ipk-gatersleben.de

T. Liiving
e-mail: liiving@ipk-gatersleben.de

S.M. Baker
e-mail: baker@ipk-gatersleben.de

membrane enveloped compartments called organelles. Only Bacteria and Archaea have prokaryotic cells and all other organisms have eukaryotic cells.

2.1.1 Cellular Organization

All cells consist of a cytoplasm surrounded by a plasma membrane, also called plasmalemma, as a border. The plasma membrane can be surrounded by a cell wall. Cells of plants, algae, and fungi have cell walls, whose main contents are cellulose or chitin. Prokaryotes have different cell wall composition than eukaryotes; a bacterial cell wall is built up mainly of peptidoglycan and an Archae cell wall of protein. Animal cells and protozoans lack a cell wall, instead they usually have some other type of covering. In most cases, animal and protozoan cells are surrounded by an extracellular matrix, which has the same basic function as the cell wall, but is more flexible. The extracellular matrix, which consists of the space between cells, is filled with polysaccharides and proteins [221]. The cytoplasm contains membrane-surrounded organelles, and the cytosol, which is the space between the organelles. Membranes in prokaryotic and eukaryotic cells are very similar and are composed of two layers of lipids into which the proteins are incorporated.

There is great variation in the size of a cell. It is organism and tissue specific and also depends on the cell's developmental stage. While most prokaryotic cells are usually up to some micrometer in diameter, eukaryotic cells can reach up to 100 μm in diameter. A typical prokaryotic cell has a simple internal structure and no membrane-surrounded organelles (see Fig. 2.1). It is thought that prokaryote cells have to remain small in order to keep the metabolism and substance diffusion in high rate. Metabolism of prokaryotes takes place largely in the cytosol and the substrates can diffuse very quickly over the cell. Organelles in a typical eukaryotic cell are the nucleus, mitochondria, the endoplasmic reticulum, the Golgi apparatus, lysosomes and peroxisomes. Usually cell organelles have flexible shape and size (about 1 to 5 μm in diameter). Some special organelles can be only found in plants, such as a

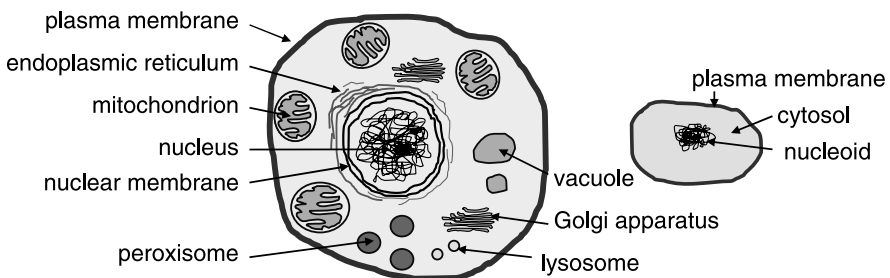
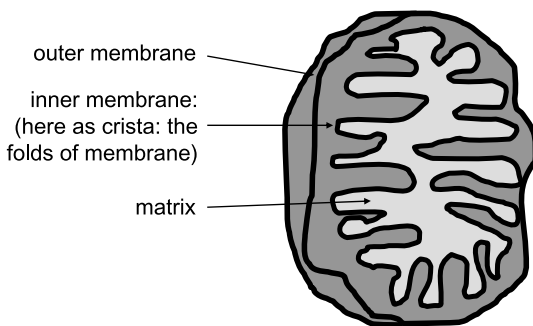


Fig. 2.1 Schematic structure of a typical eukaryotic (*left*) and prokaryotic cell (*right*). The eukaryotic cell contains the cytosol, which is surrounded by a plasma membrane and in turn contains membrane-surrounded organelles. The typical prokaryotic cell does not contain differentiated organelles

Fig. 2.2 Simplified structure of a mitochondrion: two membranes surround the matrix, in which the main metabolic steps occur. The inner membrane forms folds called cristae, which plays an important role in ATP synthesis



big central vacuole and plastids. In the next sections, the central role of mitochondria and plastids will be discussed.

For a deeper understanding of cell biology, the reader might refer to detailed textbooks [6].

2.1.2 Mitochondria

In any organism, energy is needed for most vital functions. Mitochondria are the main place for energy production in all eukaryotic cells and there are usually several hundred mitochondria in one cell. A mitochondrion is an approximately 0.5 to 1 μm long organelle, surrounded by two layers of membranes. The inner membrane is usually folded, these folds are called *cristae*. Inside of mitochondria there is a cytosol like compartment called the *matrix*.

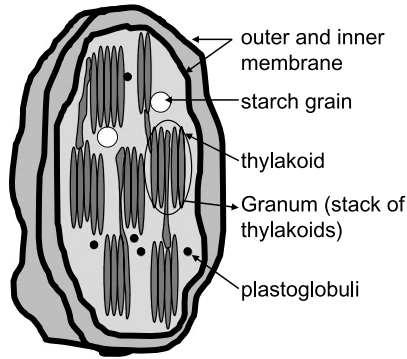
The chemical reactions in an organism usually take place in the presence of adenine triphosphate (ATP), which contains chemical energy in the form of covalent bonds between phosphates. ATP consists of one adenine, one ribose (sugar) molecule, and three phosphate groups, which can be detached to provide energy for other chemical reactions. Most of the ATP is generated in mitochondria; plant cells are an exception, in which the ATP can also be produced in chloroplasts when exposed to light. Nicotine adenine dinucleotide phosphate (NADP), which is a reducing agent important for many metabolic reactions, is also produced in the mitochondrion.

Beside the production of ATP the mitochondria has many other functions in cellular metabolism. Important metabolic pathways located in mitochondria are the citric acid cycle and oxidative phosphorylation. Mitochondria participate in the metabolism of several essential substances like amino acids, steroids, heme groups, and iron-sulphur (Fe-S) clusters. Furthermore, heat production and storage of calcium ions are regulated by mitochondria.

Figure 2.2 describes a very simplified picture of mitochondria.

For better understanding of the mitochondria and its functions, the reader is referred to recent review articles of [235].

Fig. 2.3 Simplified structure of chloroplast: a chloroplast is surrounded with two membranes; contain chlorophyll containing thylakoids, which form granum; chloroplast stroma usually contains plastoglobuli and some starch grains



2.1.3 Plastids

Plastids are organelles exclusively found in the plant kingdom. Plastids are crucial to plant functionality and develop from proplastids to generate different plastid forms. Proplastids are generally much smaller than derived plastids and do not contain pigments (e.g., chlorophyll). Several metabolic pathways take place only in plastids, such as synthesis and storage of starch, and synthesis of some pigments (e.g., carotenoids). Undoubtedly, the most important function of plastids is the process of photosynthesis, which takes place in special plastids, called chloroplasts. By photosynthesis, light energy is converted to chemical energy. Therefore in addition to mitochondria, plastids are an important place for energy production in plants.

Depending on their morphology and function, plastids have the ability to differentiate, or redifferentiate. Differentiation therefore depends on the developmental stage of the organism, the specific tissue and on environmental impulses. Plastids can be grouped to chloroplasts, chromoplasts and leucoplasts according to their main functions and the accumulating of substances. Leucoplasts contain no pigments and to this group belong amyloplasts (starch accumulation), elaioplasts (oil accumulation), proteinoplasts (protein accumulation) and combinations of these. Number and size of plastids in a plant cell are similar to mitochondria, that is, up to several hundred plastids each with a size of some micrometers. Plastids are surrounded by two membranes called the *envelope*.

In chloroplasts (see Fig. 2.3), photosynthesis takes place and therefore they are present in all photosynthetic tissues and organs such as leaves, green stems, cotyledons and hypocotyls, unripe fruits as well as seed coats and embryos. In one photosynthetic cell, there can be a few to hundreds of chloroplasts. Similar to mitochondria, in chloroplasts the inner membrane is folded forming structures called *thylakoids*, to which chlorophyll is bound. Here, the process of harvesting light energy takes place. Inside of the plastids there is a cytosol like compartment called *stroma*, in which the actual carbon fixation from carbon dioxide and synthesis of the basic units for carbohydrate takes place.

Chromoplasts are red-, orange- and yellow-colored plastids containing relatively high levels of carotenoid pigments. Carotenoids are located also in chloroplasts,

where they play an essential role by stabilizing chlorophyll. Chromoplasts often develop from chloroplasts, but may also be formed from proplastids and amyloplasts.

Amyloplasts are plastids specialized for storage starch accumulation and are found in roots, tubers, seeds, and other storage tissues.

For further reading about plastids, we refer the reader to detailed textbooks [40, 51].

2.2 Metabolism

Metabolism comprises the set of reactions that occur in living organisms for the production and degradation of organic compounds needed for an organism's vital functions. Metabolism is essential for life and transforms the input substrates into required products.

Metabolism can be divided into two categories: catabolism and anabolism. Catabolism breaks down organic matter to harvest energy in cellular respiration and also thereby can produce substrates for anabolic reactions. The chemical energy produced is in the form of ATP. Anabolism is the process of building new compounds from the basic units, which requires energy input from the outside or from catabolic reactions.

Cell metabolism is usually divided into primary and secondary metabolism. Primary metabolism can be defined as all of the processes essential for growth and development of an organism. Primary metabolism is considered a complex network of carbohydrate, fatty acid, protein and nucleic acid metabolic pathways and is largely similar in all organisms. Carbohydrate metabolism and energy metabolism may be called as a central metabolism, because they involve the production of basic structures for the other metabolic pathways. Primary metabolism also supplies the substrates for secondary metabolism. Secondary metabolism in contrast is a term for pathways that produce metabolites not absolutely essential for survival of the organism (see also Sect. 2.2.2).

2.2.1 Metabolic Pathways and Networks

The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed into another by the help of enzymes. Enzymes are crucial to metabolism because they allow organisms to drive desirable but thermodynamically unfavorable reactions by coupling them to favorable ones and by lowering the activation energy. Pathways may differ between organisms, but some basic metabolic pathways are conserved between different organisms.

Many metabolic pathways are linear, that is, they begin with a specific substrate and end with a specific product. Some pathways, such as the citric acid cycle, are cyclic, that is, the end product can be again used for starting this specific metabolic pathway. Metabolic pathways usually have several chemical reactions,

which means they generate several intermediates (metabolites which are not end product of the pathway) and the metabolic pathway can be connected to other pathways through these intermediates. Furthermore, pathways are connected to each other as a metabolite from one pathway can be a substrate for the next pathway. A collection of pathways is called the metabolic network, which is relatively dense due to many connections between different pathways.

2.2.2 Metabolites

A metabolite is a small molecule produced within the cell and participating in metabolic reactions. A primary metabolite is essential for growth, development, and/or reproduction of an organism. Amino acids are primary metabolites being the basis for proteins and relevant for many biochemical processes. Many important primary metabolites belong to the class of sugars like glucose and fructose, or contain sugar chains. A secondary metabolite has some other, less vital function. Examples of secondary metabolites are antibiotics, pigments, and hormones.

2.2.3 Enzymes

As most chemical reactions are relatively slow, there is a need for catalysts. Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions without being consumed, and are therefore essential for metabolism. Almost all processes in a biological cell need enzymes to occur at significant rates. Like all catalysts, enzymes work by lowering the activation energy (the energy that is required to activate a process) for a reaction, thus dramatically increasing the rate of the reaction. Most enzyme reaction rates are orders of magnitude faster than those of comparable spontaneous reactions.

Enzymes are selective for their substrates and speed up only up to a few reactions. The set of enzymes produced in a cell determines which metabolic pathways occur in that cell. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell. An enzyme-catalyzed reaction starts by binding the substrate at a special place of the enzyme, called the active site. The active site usually is shaped in a particular way to allow interactions with the substrate, which results in binding of the substrate [212]. In many cases, the substrate also changes shape slightly as it enters the active site. After the enzyme has catalyzed the reaction, the new product is released. Figure 2.4 describes this process.

In a cell, chemical reactions can be regulated by several enzymes. Enzymes that catalyze the same chemical reaction but differ in their amino acid sequence are called *isozymes* (also *isoenzymes*). In some cases, slightly different enzymes are formed from one gene, in this case the two proteins are called *isoforms*. Both

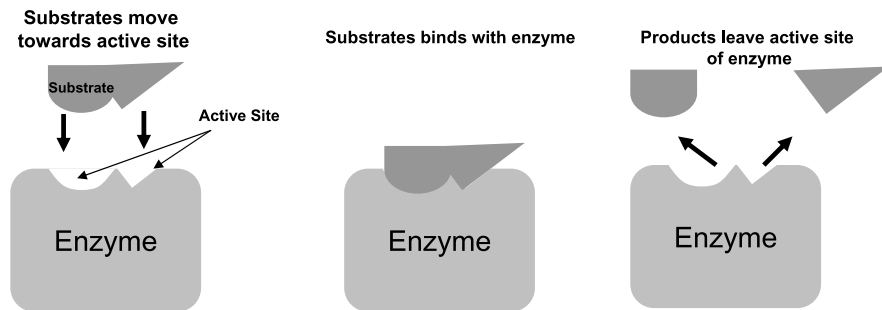


Fig. 2.4 Basic working process of an enzyme. The substrate binds to the enzyme in a site called the active site. After binding of the substrate, the enzyme catalyzes the reaction to create the product. The product is then released from the active site

isozymes and isoforms may display different kinetic parameters and/or regulatory properties. The parallel presence of isozymes/isoforms is needed to regulate the cell metabolism according to the needs of a given tissue or organ and/or to meet the needs of a developmental stage. Furthermore, different isozymes might be targeted to different cell compartments (cell organelles or cell parts) and thus allow compartment-specific regulation of the respective enzyme activity.

Enzymes can act alone or with the help of several factors, such as metal ions or organic molecules (called cofactors). The velocity of enzymatic reactions is affected by other molecules, environmental conditions, as well as the substrate and product concentrations. These regulatory factors are called inhibitors or activators, depending on the direction of the regulation (up or down).

2.2.4 Enzyme Inhibition

Molecules which bind to the enzyme and thereby decrease its activity are called inhibitors. Inhibition can be reversible or irreversible, depending on the inhibitor. The major role in regulation of synthesis may lay in reversible inhibition, in which enzyme activity is the same after removal of the inhibitor. There are basically three types of inhibition: competitive, noncompetitive and uncompetitive inhibition.

In competitive inhibition, the substrate has to compete with the inhibitor for binding to the active site. Commonly, the chemical structure of the competitive inhibitor resembles the chemical structure of the substrate. The degree of inhibition of the specific reaction in the cell depends on substrate and inhibitor concentrations. The principle of competitive inhibition is outlined in Fig. 2.5.

In noncompetitive inhibition, the inhibitor binds to some place other than the active site of the substrate in the enzyme and reshapes the enzyme in a way that the active site for a substrate is changed, and thus the substrate can not bind to the enzyme (see Fig. 2.6). In other words, noncompetitive inhibition can be described as

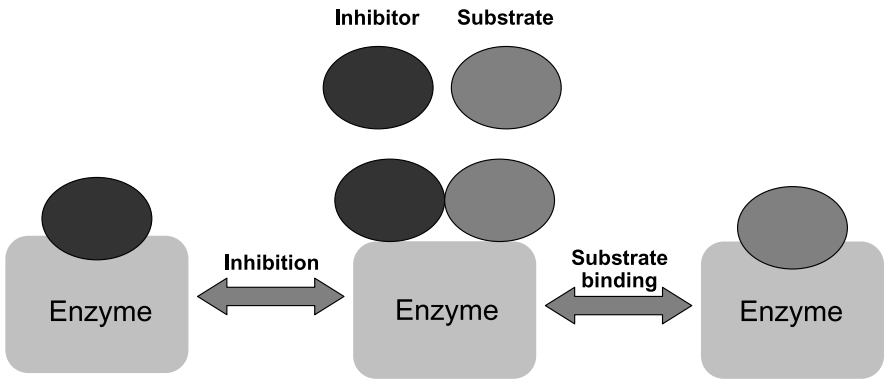


Fig. 2.5 Working procedure of competitive inhibition. In this type of inhibition, both the inhibitor and the substrate compete for the same active site of the enzyme. Both of them have the capability to bind with the enzyme in the active site. This creates a competitive environment between the substrate and the inhibitor to bind with the enzyme

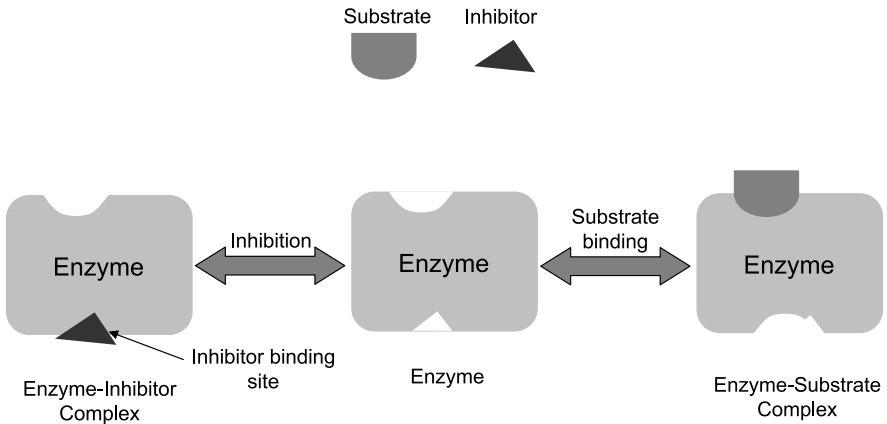


Fig. 2.6 Working process of noncompetitive inhibition. In this type of inhibition, the inhibitor binds to some place other than the active site. This place is called the allosteric site. By binding to the allosteric site, the inhibitor changes the structure and shape of the enzyme, so that the substrate can no longer bind properly with the enzyme and thus reduces the maximum rate of the chemical reaction

allosteric inhibition. Allostery (from the Greek “other site”) means that the regulatory binding site of the inhibitor and the substrate binding site (the active site) are physically separate.

An uncompetitive inhibition means that the inhibitor binds to its regulatory site in the enzyme after the substrate has bound to the active site forming an inactive substrate-inhibitor-enzyme complex. For a deeper understanding of enzyme inhibition, the article [324] is suggested.

2.2.4.1 Enzyme Kinetics and Activity

Enzyme activity describes the use of the substrate or the amount of formed products. The SI unit for enzyme activity is katal (kat), which is the conversion of one mole substrate into a new product in one second (mol s^{-1}). The activity can also be expressed in enzyme units (U), which describe the amount of the enzyme that catalyzes the conversion of one micro mole of substrate per minute ($1 \text{ U} = 1 \mu\text{mol min}^{-1}$).

$$1 \text{ U} = \frac{1}{60} \text{ micro katal} = 16.67 \text{ nano katal}$$

The term enzyme kinetics refers to the study of the speed, also called rate or velocity, of an enzyme-catalyzed reaction. Reaction velocity depends on the substrate concentration as well as the environment, especially on temperature, pressure and pH value of the surrounding medium. The velocity of enzymatic reactions describes the speed of the reaction, which is composed of 3 steps: substrate binding to the enzyme, the process of production of the new product, and release of the product. The following equation describes this process in a simplified way:



The equation describes an irreversible reaction in which E is the enzyme, S is the substrate, ES is the enzyme-substrate complex, and P is the product.

The reaction velocity v is equal to the rate of formation of P or the rate of reduction of S. The following equation expresses the relationship between the reaction velocity and the change of concentration of substrate and product with time.

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$$

A graph of product concentration vs. time is given in Fig. 2.7. The time-dependent behavior of the product concentration can be divided into three stages.

It is difficult to fit a curve to a graph of product as a function of time, as it ignores the transient phase and assumes that the reaction is irreversible. Therefore, the enzyme velocity is typically described as a function of substrate concentration as depicted in Fig. 2.8.

The simple velocity function shown above can be described by the Michaelis-Menten equation

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

K_m (Michaelis-constant) is (roughly) an inverse measure of the affinity, i.e. the strength of binding between the enzyme and substrate. The lower the K_m , the greater is the affinity, which means that lower concentrations of substrate are needed to achieve a certain velocity of the turnover of substrate (the amount of product produced per unit time). K_m is measured as the substrate concentration at half of maximum velocity ($v_{\max}/2$). Maximal velocity is the maximum rate of the reaction, which occurs when the enzyme is completely saturated with substrate.

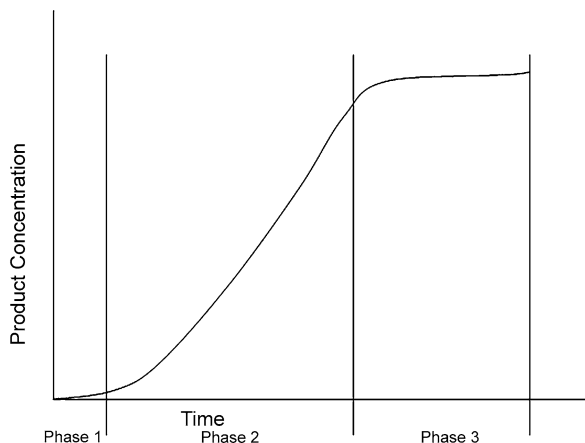
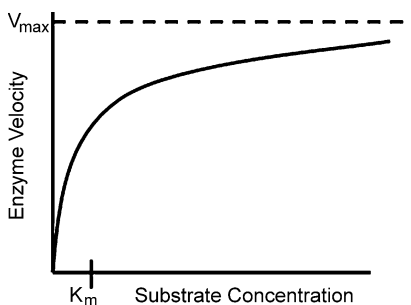


Fig. 2.7 Time-course of product formation in a typical enzymatic reaction. The graph shows the change of product concentration over time. With the passing of time, the rate of product accumulation also increases. This product accumulation can be divided into three phases. At the start of the reaction, the product concentration is relatively low (phase 1). Then for an extended period of time, the product concentration increases in a nearly linear manner with time (phase 2). During the last periods the enzyme is saturated, so the curve starts to level off (phase 3)

Fig. 2.8 Substrate concentration versus reaction rate of a typical enzymatic reaction. V_{\max} is the maximal possible reaction rate. K_m is the substrate concentration at which the reaction reaches $1/2V_{\max}$. This graph can be described with the Michaelis–Menten equation, see text



Inhibition has an effect on the v_{\max} and/or K_m values. For *competitive inhibition*, in the presence of an inhibitor, a higher substrate concentration is required to achieve the same velocities that were reached in its absence. So while v_{\max} can still be reached if sufficient substrate is available, one-half v_{\max} requires a higher $[S]$ than before and thus the apparent value of K_m is larger than without inhibition. For *noncompetitive inhibition*, enzyme molecules that have been bound by the inhibitor are taken out of the reaction so that the reaction rate is reduced for all values of $[S]$, including v_{\max} and one-half v_{\max} , but K_m remains unchanged because the active site of those enzyme molecules that have not been inhibited is unchanged. For further reading on enzyme kinetics, the reader is referred to a textbook [38].

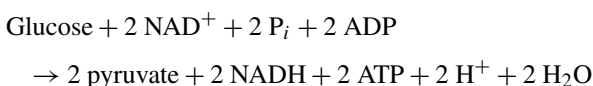
2.2.5 Central Metabolic Pathways

Central metabolic pathways are usually considered as pathways for the synthesis of carbohydrates, proteins and fatty acids. These are essential pathways as they produce energy and metabolites which are the starting point for many further products. Common central metabolic pathways are glycolysis, the citric acid cycle, the pentose phosphate pathway, and fatty acid synthesis, each of which will be described in detail below. Central metabolic pathways can be differently defined for plants and animal cells, or for heterotrophic and autotrophic cells. Autotrophic cells can produce organic substances from nonorganic elements (e.g., photosynthesis in plants). Heterotrophic organisms or cells can not produce organic substances from nonorganic elements and are therefore dependent on autotrophs. Plants are *mixotroph organisms*; they contain both, autotrophic and heterotrophic cells.

2.2.5.1 Glycolysis

Glycolysis is an essential part of energy generation in a cell and takes place in the cytosol. Glycolysis is the metabolic pathway that converts glucose, which is the main energy source of most of the organisms, into energy. The energy released in this process is used to form the high energy compound ATP and the reductant NADH (reduced nicotinamide adenine dinucleotide). In addition, the end product pyruvate is a relevant starting point for many other metabolic pathways.

The overall process of all steps of glycolysis is:



For a more detailed introduction into glycolysis, the reader might refer to the review article of [305].

2.2.5.2 The Citric Acid Cycle

The citric acid cycle, also named tricarboxylic acid (TCA) cycle or Krebs cycle, is important as an energy generator, and it is involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water. In eukaryotic cells, the citric acid cycle takes place in the mitochondrial matrix. Pyruvate, which is the end product of glycolysis, is transported from the cytosol into the mitochondrial matrix. There it is transformed by the enzyme pyruvate dehydrogenase under addition of Coenzyme A into acetyl-CoA and CO₂. Coenzyme A carries a thiol group and reacts with carboxylic acids to form thioesters, thus functioning as an acyl group carrier. Acetyl-CoA is the primary substrate entering the citric acid cycle.

The citric acid cycle can be called the second step in respiration. The citric acid cycle oxidizes acetyl-CoA to carbon dioxide, and produces energy carriers like ATP and GTP (guanosin triphosphate, generated in animal cells) and molecules relevant

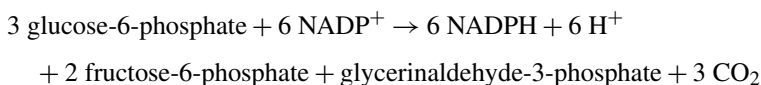
to redox reactions, such as NADH. Beside that the intermediates of the citric acid cycle have a big relevance as they are substrates for many other biosynthetic pathways, for example, the synthesis of several amino acids.

In a process called oxidative phosphorylation, the reducing equivalents that are generated from TCA cycle activity are used by the electron transport chain to drive the synthesis of ATP. For this, the protons and electrons from NADH are transported at the mitochondrial inner membrane. The resultant potential across the membrane is used to drive ATP synthesis.

2.2.5.3 The Oxidative Pentose Phosphate Pathway

The oxidative pentose phosphate pathway (OPPP) has the role to produce the reductant NADPH and metabolic intermediates for several biosynthetic pathways including synthesis of nucleotides, aromatic amino acids, and phenylpropanoids. In most cells, the OPPP takes place in the cytosol, but in plants it is mainly localized in plastids. In plants, NADPH is also synthesized by photosynthesis, the OPPP is only essential for nonphotosynthetic cells.

The overall sum reaction of oxidative pentose phosphate pathway is:



For a better understanding of the role of OPPP, the review of [214] is suggested.

2.2.5.4 Fatty Acid Synthesis

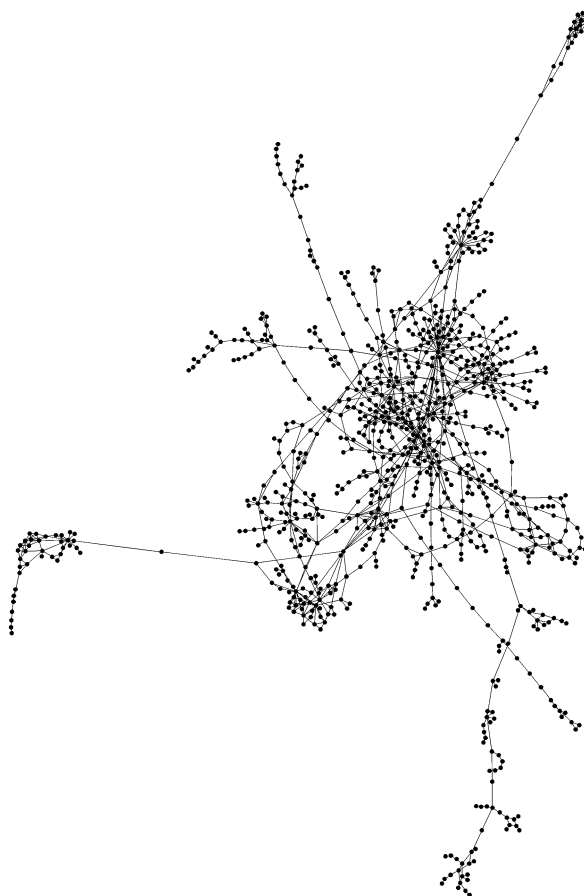
Fatty acids are usually synthesized in the cytosol. However, in plants their synthesis also takes place in the chloroplast stroma in photosynthetically active cells. Fatty acids constitute an energy storage form, but also play an important role in cell structure as a membrane compound. Storage lipids are formed by esterification of glycerol with up to three fatty acids.

Fatty acids are synthesized from acetyl-CoA, which in eukaryotic cells is synthesized in the mitochondrion, and thus has to be transported to the cytosol in order to be available for fatty acid synthesis. This is achieved by exporting citrate to the cytosol and cleaving it into oxalacetate and acetyl-CoA.

2.2.6 Metabolic Networks

The functions of a living cell are regulated by different networks of interacting biochemical components. How these molecules are connected to each other and what are their influences on the activity of each of the reactions under diverse physiological conditions is a central issue in understanding cellular organization. As mentioned

Fig. 2.9 Schematic view of a metabolic network. The nodes in the network are the metabolites and the edges between them are the enzymes. The figure was created using the software VANTED (Visualization and Analysis of Networks containing Experimental Data) [177]



before, the metabolic pathways can be connected to each other and produce starting substrates or intermediates for other metabolic pathways. All the pathways together form a large metabolic network. This metabolic network comprises the chemical reactions of metabolism as well as the regulatory interactions that guide these reactions. Figure 2.9 shows a major component of the metabolic network in the model plant *Arabidopsis thaliana*.

2.2.7 Regulation of Metabolism

A cell contains a large number of molecules. To ensure that every molecule is produced in the correct amounts at the time required, the cell must have a control device for their production and consumption. Metabolism is regulated through several factors, which can be both source-based and environment-based (temperature, light). In addition metabolism also depends on the developmental stage of the organism

as well as the tissue function. This means that the current needs of the cell influences the metabolism. In the simplest case, the regulation of a metabolic reaction is achieved by controlling two issues: (1) the availability of enzymes or (2) the activity of the enzyme. More complicated mechanisms of metabolic regulation comprise, but are not limited to, redox regulation, allosteric regulation, and feedback mechanisms.

2.3 Gene Expression

Gene expression is the mechanism by which proteins are produced from DNA (deoxyribonucleic acid). The main role of DNA in the cell is the storage of long-term information. DNA contains literally the information needed to construct and maintain all components of cells, as well as to mediate all regulatory processes. DNA is a molecule composed of a chain of four different types of nucleotides (also called bases) named adenine (A), thymine (T), guanine (G), and cytosine (C). DNA has a double helix structure and each strand runs opposite to the other which makes them anti-parallel. The backbone of the DNA consists of the sugar ribose and phosphate groups.

Different parts of DNA chain have various roles. The DNA segments that carry the genetic information are called genes. Other segments of DNA sequences have structural purposes, or are involved in regulating the use of this genetic information.

The whole process of gene expression can be simply divided into two major stages: transcription and translation (see Fig. 2.10).

2.3.1 Transcription

By transcription, RNA is generated from DNA. RNA is the abbreviation of ribonucleic acid, which is a single stranded long chain of nucleotides. Instead of the base thymine it contains the base uracil (U).

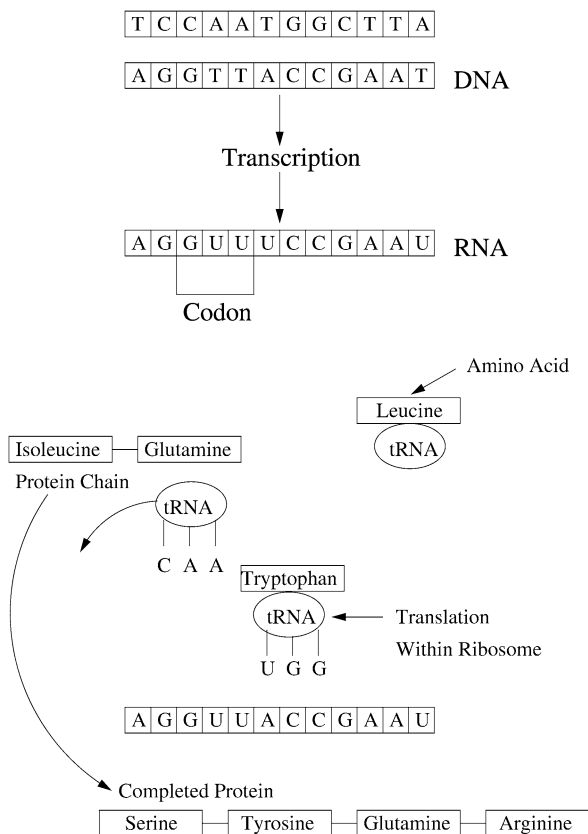
Transcription is the process of the transfer of genetic information from the archival copy of DNA to RNA. The gene sequence is copied to produce a complementary nucleotide RNA strand called messenger RNA (mRNA), as it carries a genetic message from the DNA to the protein-synthesizing machinery (ribosomes) of the cell.

Transcription is a highly regulated process which is guided by the enzyme RNA polymerase. Transcription is regulated by transcription factors which suppress or promote binding of the RNA polymerase onto a specific DNA sequence. Transcription factors are short protein sequences and affect the transcription by binding to DNA.

2.3.2 Translation

Translation is the second large stage in gene expression. In this stage peptides, that is, amino acid chains, are created by decoding the information contained in the

Fig. 2.10 Basic steps of gene expression. This is divided into two phases, transcription and translation. In transcription, double stranded DNA is converted in to a single stranded RNA. In translation, the RNA enters the ribosome, where it encounters the complementary codon of tRNA (transfer RNA) that carries the amino acid. Amino acids then connect with each other to create a peptide chain which is called protein



mRNA. It is mediated by the ribosomes which are located in the cytosol. This process uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. In this process, the tRNA (transport RNA) carrying one amino acid per molecule is transported to a protein complex called the ribosome. In the ribosome, the tRNA binds specifically to a triplet of three mRNA nucleotides, which is called the codon. This is mediated by the fact that each tRNA contains an anticodon that matches a specific codon. The amino acids carried by the tRNA are joined together as a chain according to the codon chain. The polypeptide chain is converted into a three-dimensional, functional protein by specific folding procedures aided by chaperones. Furthermore, different or identical proteins can bind together to form protein complexes.

There are $4^3 = 64$ different codon combinations possible with a triplet codon of three nucleotides, but there are only 20 amino acids to code for. Thus, multiple codons might be used to encode the same amino acid. Furthermore, in the mRNA there is one start codon (AUG), which specifies the position where the peptide synthesis starts, and three stop codons (UAA, UAG, or UGA), which specify the end of peptide synthesis.

2.3.3 Gene Regulation

While some housekeeping genes are expressed all of the time and in all cells, most genes are turned on or off in a specific manner. These genes are specific to different tasks and are only required at a specific time and in specific cell types. Furthermore, some genes are expressed in certain tissues only at a certain developmental stage of the organism.

For example, the arrival of a hormone may turn on (or off) certain genes in that cell. So producing a protein at a wrong place or at a wrong time will disrupt the whole mechanism. Genes are turned off by transcription factors if there is no need for the protein that they encode or turned on when the environment changes and the proteins are once again needed. These mechanisms prevent a waste of energy.

2.3.4 Gene Regulatory Networks

Gene (or genetic) regulatory networks (GRNs) regulate the interactions between genes and more precisely the expression of genes in specific amounts and at a specific time and place. Very simplified, a GRN consists basically of a signaling pathway, a target gene and gene products. The studies of GRN therefore are focused on gene transcription to RNA, translation and protein formation as well as the interactions of these processes and products.

GRNs can be divided into many types, but two of them are basic: transcription factor network and gene expression network. The transcription factor network involves the regulatory mechanism which affects the first step of gene expression: the transcription of DNA into RNA. This network consist of transcription factor genes, transcription factors, and of regulatory molecules which regulate the transcription factor binding to a special DNA sequence called the promoter. The transcription factor network is regulated by external and cellular signals. Under gene expression, the network is meant to produce the functional gene product. The mRNAs and proteins are products of gene expression. These products can interact with each other as well as with transcription factors and therefore belong to a gene regulatory network.

For further reading and understanding of GRNs and the challenges of investigating GRNs, there are several reviews like [13, 181] and [247].

2.4 Signal Transduction

A cell has to react to the changes taking place inside itself, in surrounding cells and outside the organism. One of the most important functions of cell signaling is to control and maintain a physiological balance (called homeostasis) within the body. The reaction is controlled by handing over the signal to a receptor which leads to reactions called signal transduction. Activation of different signaling pathways

leads to diverse physiological responses, such as cell proliferation, cell death, cell differentiation, and changes in metabolism.

Signals can be molecular as well as sensory in response to environmental changes such as light, pressure and temperature. Molecular signals can be simple elements, usually in the form of ions, complex inorganic as well as organic molecules. The start signal is called the stimulus, effector or elicitor. The signals are received by receptors specific to the signal and these receptors pass this message to a messenger.

There are many different signal transduction pathways which involve enzymatic reactions to activate the correct response. Signaling pathways in cells interact with each other, as there are many signals received at the same time. The signaling proteins and secondary messengers inhibit or increase the signal transduction or gene expression.

Signal transduction pathways are different depending on the signal and the receptor and usually consist of several steps of transferring the information. Usually, one signal can mediate many reactions; this is called a signal-transduction cascade, through which the response reactions can be regulated more precisely. In this process, the signal is passed over from one signaling protein to another. Signal transduction ends with a molecule which can activate the gene expression. This could be a transcription factor, by which many genes can be activated at the same time.

The term signal transduction network refers to a complex of all reactions (including interactions) in signaling from receptors to final targets that mediate the specific gene expression.

One of the best known signaling transduction systems is the mitogen activated protein kinase (MAPK) signaling pathway [85], which regulates many genes and therefore is important for several cellular processes and development. The MAPK functions as a cascade of kinases: one kinase phosphorylates (adds phosphate) the next kinase in order to propagate the signal and the last kinase phosphorylates the target protein.

In a cell there are four main groups of proteins involved in signal transduction: protein kinases, protein phosphatases, guanosine triphosphatases, and adapter proteins. Kinases and phosphatases regulate giving over the signal by adding or removing phosphate, respectively. The adapter proteins act as linkers or binders.

A clear understanding of the signal transduction pathways and the signal transduction networks is hard to achieve, because there are many participants and cross talk between the transduction pathways as well as between gene expression levels. The problem of understanding signaling pathways is well reviewed by [102].

2.4.1 Hormones and Other Signaling Molecules

There are many internal and external signaling molecules, which belong to different chemical groups. Hormones belong to signaling molecules, which are transported from one cell to another. The main role of the hormones is to regulate the growth

and development of an organism. Many hormones belong to the chemical group of steroids, which is a very common group of signaling substances in mammals. In plants, there are hormone like substances called phytohormones. Furthermore, single elements like calcium or potassium play an important part of the cell signaling system. Signaling with ions is usually based on changes in the cell's electrical potential and concentration of ions.

Most external signals can not penetrate the cell, these are typically large molecules such as proteins, peptides and amines. Some signals which can penetrate the cell are light, steroids, gases, some hydrophilic molecules, and ions.

2.4.2 Receptors

Receptors are proteins specialized to detect signals and are usually membrane bound. Signals can enter the cell through the plasma membrane bound receptor proteins, ion channels, diffusion, or by active transport through the plasma membrane with the help of transport proteins.

The receptor usually gets modified by the signal and this conformation initiates signal transduction. Receptors can generate a chemical signal inside the cell by interacting with one or more proteins.

2.5 Problems

2.1 What is the role of mitochondria and plastids in a cell?

2.2 What is metabolism? Describe different categories of metabolism.

2.3 What is the role of metabolites and enzymes in a metabolic network?

2.4 How do enzymatic reactions take place?

2.5 What is described by the Michaelis-Menten equation?

2.6 What are common central metabolic pathways? Describe the role of central metabolic pathways.

2.7 What are the processes that take place in a gene expression?

2.8 Describe in your own words the role that a receptor could play for metabolism.

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